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MICROBIOLOGICAL ESTIMATION OF ANTIFUNGAL BLOOD LEVELS FOLLOWING ADMINISTRATION OF FUNGICIDES [1]

J. E. TARBET AND T. H. STERNBERG [2]

(WITH 2 FIGURES)

In recent years, a number of new compounds exhibiting antifungal properties have been discovered (**2**, **3**, **4**, **5**, **7**). Some of these have been found to possess *in vivo* effectiveness in mycotic disease (**1**, **6**, **8**, **9**, **10**), thus creating a need for suitable techniques for determining specific antifungal "levels" in body fluids and tissues. To date, a number of such techniques has been described, but all have certain limitations. Taplin *et al.* (**12**) have measured prodigiosin in plasma of rabbits by spectrophotometric analysis following chemical extraction. Christie *et al.* (**1**) have used colorimetry for ethyl vanillate in blood. Saltzman (**11**) has devised a fluorimetric estimation for stilbamidine in mixtures of stilbamidine with blood and urine. In reporting the discovery of ascosin, an antifungal antibiotic from *Streptomyces canescus*, Hickey and associates (**5**) included information on plasma levels obtainable by serial dilution and turbidimetry with *Saccharomyces cerevisiae* after intraperitoneal injection of ascosin in mice and rabbits.

The present report describes a microbiological method for the estimation of serum concentrations of antifungal substance utilizing *Candida*

[1] From the Medical Service, Veterans Administration Hospital, and the Division of Dermatology, Department of Medicine, University of California School of Medicine at Los Angeles.

[2] The authors wish to acknowledge the valuable technical assistance of Midori Oura.

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tropicalis (Castellani) Berkhout as the test organism. The results of assay of the growth of *C. tropicalis* in dilutions of guinea pig blood serum obtained after the administration of various antifungal drugs and other substances are presented. Evaluation of the reproducibility and specificity of the assays is made and the results are compared with those obtained by ordinary turbidimetric, paper disk, or plate dilution methods.

MATERIALS AND METHODS

Drugs. Serum pools were obtained from animals following the injection of each of the following substances: actidione,[3] ascosin,[4] aureomycin hydrochloride,[5] candicidin,[6] o-dichloroacetyloxine,[7] 17-hydroxycorticosterone-21-acetate (Compound F),[8] ilotycin,[9] 3-keto-21-(piperidyl)Δ-4-pregnadiene monohydrobromide,[7] nystatin,[10] physiological saline solution, polyvinyl pyrollidone, pregnenolone,[11] prodigiosin,[12] rimocidin,[13] and stilbamidine isethionate.[14]

PREPARATION OF TEST SERA. For each of the drugs except prodigiosin, 16 milligrams per kilogram were administered intraperitoneally in one milliliter volumes of physiological saline solution to male guinea pigs of approximate weight 700 grams. With prodigiosin, a suitable volume of a suspension of three milligrams per milliliter of prodigiosin in 3.5 percent polyvinyl pyrollidone was injected so that each animal received 16 milligrams per kilogram. Polyvinyl pyrollidone and physiological saline solution were given in one milliliter injections as controls.

For each antifungal drug or control, including the non-fungicides, aureomycin hydrochloride, ilotycin, pregnenolone, and 17-hydroxycor-

[3] The Upjohn Company, Kalamazoo, Michigan. Trademark for the antibiotic, cyclohexamide, from *Streptomyces griseus.*

[4] Commercial Solvents Corporation, Terre Haute, Indiana. An antibiotic from *Streptomyces canescus.*

[5] Lederle Laboratories Division, American Cyanamid Company, New York, N. Y.

[6] Furnished to the authors by Dr. Hubert Lechevalier, New Jersey Agricultural Experiment Station, Rutgers University.

[7] Ciba Pharmaceutical Products, Incorporated, Summit, N. J.

[8] Merck and Company, Inc., Rahway, N. J.

[9] Eli Lilly and Company, Indianapolis, Indiana.

[10] E. R. Squibb and Sons, New York, N. Y. An antibiotic from *Streptomyces noursei.*

[11] Schering Corporation, Bloomfield, N. J.

[12] Prepared by Ralph Pressman, Ph.D., Antibiotics Research Laboratory, Research Division, Veterans Administration Center, Los Angeles, California.

[13] Charles Pfizer and Company, Inc., Brooklyn, N. Y. Trademark for an antibiotic from *Streptomyces rimosus.*

[14] William S. Merrel Company, Cincinnati, Ohio.

ticosterone-21-acetate, blood from two or more animals was obtained by means of cardiac puncture from 2½ to 3½ hours after injection. The sera from each group of guinea pigs were pooled and frozen. The pooled sera were kept in the freezer until assayed.

Pools of frozen serum from twelve or more normal guinea pigs were utilized in preparing the dilutions of the test sera for inclusion in the assay cultures and for control purposes.

Assay Method. The strain of *Candida tropicalis* employed as the indicator microorganism has been used by Tarbet, Oura, and Sternberg (**13**) in comparing the *in vitro* potencies of a variety of chemical fungicides. A culture of this fungus is maintained in the collection of the Department of Microbiology at Rutgers University. The procedures followed in the present work were similar to those previously reported. The principal modification was in the composition of the assay culture medium in order to adapt it to the requirements of measuring fungistats in mixtures of serum. The medium was composed as follows:

Asparagine	0.5 gm
Dextrose	2.0 gm
$MgCl_2 \cdot H_2O$	0.2 gm
Phosphate buffer,[15] pH 6.4	20.0 gm
Distilled water	80.0 gm

Cultures were prepared in one milliliter volumes containing 0.5 ml of this synthetic broth and 0.5 ml of guinea pig serum. Each test was prepared in triplicate in serological tubes (13 × 100 mm). For inoculation of the assay cultures, a suspension in broth of *C. tropicalis* from 15-day-old growth on Difco Sabouraud's dextrose agar slants was obtained by washing the cells from the surface of the slant with broth, followed by vigorous agitation of the cell suspension. With 15-day-old cultures and sufficient agitation, this resulted in a suspension of unicellular forms.

The density of the inoculum was adjusted by means of direct microscopic counts of the fungous cells, employing the leukocyte chambers of the Levy hemocytometer. In each experiment the inoculum was adjusted to contain 250 ± 25 cells per cubic millimeter. Two hundred and fifty cubic millimeters of this inoculum were incorporated in each of the assay cultures.

Incubation of the cultures was carried out at 27° C with constant shaking (Kahn type shaker: 275–285 oscillations per minute through 1½" stroke). When the fungous growth in control cultures containing broth

[15] Sorensen Phosphate Standards. *Practical Physiological Chemistry.* P. B. Hawk and O. Bergheim. 11th Edition. The Blakiston Company, Philadelphia, 1944.

and normal serum only measured 1750 or more budding fungi per cubic millimeter (15 to 18 hours of incubation), all cultures were immediately transferred to a freezer in order to stop further changes and arrest growth. When the temperature of the cultures reached − 2° C, they were removed to ordinary refrigerator temperature (4° C) until the numbers of blastospores could be determined.

Prior to counting the blastospores, each culture was agitated for 30 seconds and the counting chambers of the Levy hemocytometer were filled with the undiluted suspension. The dose-response of the microorganism, as reflected in the number of blastospores in serial cultures containing increasing known amounts of test drug, was found by direct cell count and taken as a standard. To estimate the specific antifungal potency of test sera, the growth of the indicator organism in cultures containing no added drug, but increasing amounts of serum from animals injected with the drug, was compared with the standard.

The differences in cultural density which occur in the sensitive, early growth stages of *C. tropicalis* could best be compared by direct cell counting. As the culture medium contained 50 percent of mixed sera, differences in growth were not distinguishable by ordinary methods of turbidimetry.

The results of direct cell counting were compared with those of photoelectric turbidimetry in preliminary experiments. The Coleman spectrophotometer was used, employing wavelength 400 millimicra. Results are tabulated below.

GROWTH OF C. TROPICALIS, MEASURED BY PHOTOELECTRIC TURBIDIMETRY AND BY DIRECT CELL COUNTS

Incubation interval (hours)	Percent transmission	Numbers of blastospores per mm^3
0	99.8	80
4	100.0	150
6	100.0	288
8	100.0	495
11	99.6	1145

The differences in cellular turbidity were obscured by the serum. The early growth changes were discernible only by the counting technique.

When serial dilution and plating of the assay cultures was employed, the resulting dose-response data were irregular, difficult to reproduce from experiment to experiment, and larger quantities of the drugs were required. Detection of fungistats in serum by paper disk or cup-plate techniques was totally unsatisfactory, except with serum samples of unusually high potency. With the cell counting technique, antifungal

activity measurable in fractional micrograms per milliliter could be observed.

Calculation of Antifungal Serum Concentrations. With a given antifungal drug, the concentration necessary to produce any particular grade of response in cultures of *C. tropicalis* can be found. The concentration required to inhibit 50 percent of the cells is the most easily determined. This dose has been termed the median effective dose, symbolized by the letters MED (**14**). For the purposes of this report, doses evoking other than the median level of response will be designated ED_{75}, ED_{95}, etc.

It should be remarked that susceptibility to antifungal agents is normally distributed in cultured spore populations. Values derived from

TABLE I

ESTIMATION OF ACTIDIONE IN GUINEA PIG SERUM FORTIFIED WITH ACTIDIONE

Dose-response in 50 percent normal serum broth		Dose-response with fortified serum (1.5 mcg per ml)			Assay
Actidione mcg per ml	Number of blastospores per 0.4 mm^3	Dilution of fortified serum	mcg per ml of actidione in diluted serum	Number of blastospores per 0.4 mm^3	mcg per ml of actidione in whole serum
0.00	1493	1–20	0.075	884	0.80–1.60
0.04	1140	1–10	0.15	553	1.00–2.00
0.08	780	1–5	0.30	372	1.50–2.00
0.10	712				
0.20	417				mean: 1.48
0.30	397				
0.40	351				
MED: 0.09 mcg per ml		Assay (from graph, Fig. 1): 1.3 mcg per ml			

the MED response of the cultured microorganism are more easily reproducible from experiment to experiment because the larger number of cells in the middle classes of susceptibility reduces the variation. ED_{95} or ED_{100} could be determined with as great precision only if many more spores were counted in each test.

In assaying the specific antifungal strength of a serum of unknown activity, MED for the drug in question was first found from a standard dose-response of the assay organism in cultures in a normal serum-broth mixture containing known added amounts of the crystalline drug. The quantity of the test serum to produce this same MED response was then ascertained. With these data the antifungal activity of the test serum could be calculated in terms of micrograms of drug per unit volume of the serum.

Example: With each drug, dose-response curves were repeatedly obtained in different sera in order to ascertain the variation in MED which might be expected. With actidione, for example, MED was found to fall between 0.09 and 0.16 mcg per ml. In assaying a serum for actidione, the standard dose-response curve, obtained as a control, was accepted if MED was found to be within this range.

Pooled guinea pig serum fortified with crystalline actidione was tested as follows (TABLE I):

An estimate of the actidione content of the fortified serum may be made directly from the data, as indicated in the right hand column

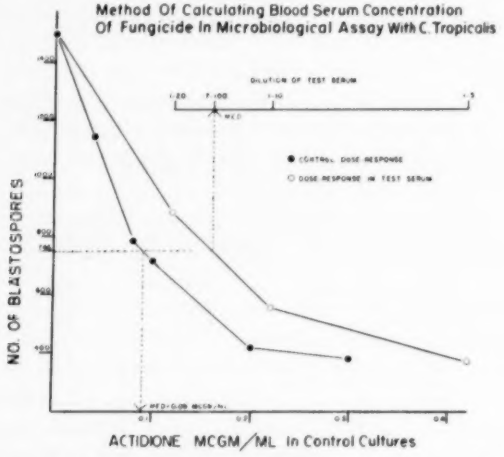

FIG. 1. Horizontal dotted line indicates MED level of growth (746 blastospores per unit volume of culture). From interpolation on control curve MED for actidione is found to be 0.09 mcg per ml (lower horizontal axis). From the parallel curve for the growth response of *C. tropicalis* to increasing concentrations of the test serum, it is seen that the dilution of the serum which induced the MED response was 7–100 (upper horizontal axis). Actidione activity of the undiluted test serum assayed $1.3 \frac{(100 \times 0.09)}{7}$ mcg per ml.

(TABLE I). The use of a graph is preferable with most drugs and simplifies the calculation. Interpolation on the standard dose-response curve (FIG. 1) indicates MED for actidione to be 0.09 mcg per ml. From the parallel curve for the dilutions of the fortified serum (FIG. 1) it appears that the dilution of this serum which induced the MED response was 7–100. Therefore, 0.07 ml of this serum possessed activity equivalent to 0.09 mcg of actidione. A simple calculation indicates a

concentration of actidione in the test serum of 1.3 mcg per ml. In this experiment to recover an added trace of actidione, recovery fell short of theoretical values by from 0.02 to 0.2 mcg per ml.

Assay of added fungicides in serum obtained following injection of physiological saline solution or polyvinyl pyrollidone was also satisfactory. No normal serum batch or test serum batch obtained after injection of non-fungicidal material was found to contain endogenous substances inhibiting the growth of *C. tropicalis*. But occasional sera were

TABLE II

RECOVERY OF ADDED NYSTATIN (8 MCG PER ML) IN SERA WITH MARKED GROWTH-STIMULATING PROPERTIES

Dose-response in 50 percent normal serum broth		Test sera fortified with nystatin (8 mcg per ml)		
			Number of blastospores per unit volume*	
Nystatin mcg per ml	Number of blastospores per unit volume*	Nystatin mcg per ml	Serum obtained after injection of ilotycin	Serum obtained after injection of pregnenolone
0.0	1000	0.0	6862	9358
1.75	987	2.0	642	606
2.0	581	2.4	543	482
2.5	385	2.8	420	365
3.0	308	3.2	379	283
5.0	143	4.0	330	164
MED (mcg per ml): 2.2			2.5	2.3
Calculated recovery of Nystatin in test sera (mcg per ml):			6.9	7.4

* To facilitate comparison, values in each series have been calculated to the corresponding volume of 50 percent normal serum broth which contained 1000 blastospores.

encountered with which the growth of the indicator fungus proceeded at rapid rates relative to the rate in normal serum.

Sera with such growth-stimulating properties were obtained from animals following injection of the non-fungicides, aureomycin hydrochloride, ilotycin, 17-hydroxycorticosterone-21-acetate, and pregnenolone. Of 35 pools of normal guinea pig serum which were tried, only one exhibited such growth-accelerating action. In eight tests of four serum batches obtained following injection of the non-fungicides, all possessed this property. Attempts to measure antifungal concentration in such sera using a normal serum standard might be expected to fall short, as

the more rapid growth in the test serum would mask the presence of endogenous or added fungicide.

For this reason, assays of fungicides in sera obtained after injection of non-fungicidal drugs were included in this study as a "check" on the specificity of the assays and on the degree of interference with the assay results which might be expected with growth-accelerating sera. Enhancement of the growth of *C. tropicalis* by sera obtained after injection of non-fungicides did not interfere significantly with the assay of added antifungal substances (TABLE II).

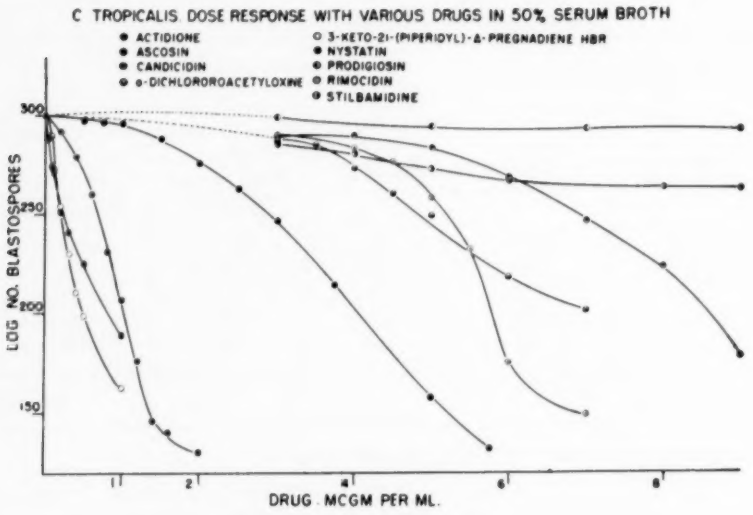

FIG. 2. Discussion in text.

EXPERIMENTAL

MED for Antifungal Drugs in Serum Medium. In FIG. 2, typical dose-response data for *C. tropicalis* with several antifungal drugs have been illustrated graphically. Higher potencies were seen with the available samples of actidione, ascosin, and 3-keto-21-(piperidyl)-Δ^4-pregnadiene monohydrobromide. The available samples of the other drugs were less potent microgram for microgram. MED for the nystatin sample was in the middle range of potencies. MED for each particular drug sample was repeatedly determined, using different serum lots. The values obtained have been listed in TABLE III. They indicate the

degree of reproducibility of the assays and represent the potencies of the particular drug samples for *C. tropicalis*.

Antifungal Serum Levels in Guinea Pigs Following Injection of Drugs. With each drug regardless of potency, 16 mgm per kg were administered intraperitoneally. Blood was taken by means of cardiac puncture 2½ to 3½ hours after injection of the drug. Sera from animals receiving the same drug were pooled and frozen as soon as separated. Portions of each serum pool were thawed and assayed in from one to seven days following freezing. Two or more assays were performed with each test serum pool.

No antifungal activity was detected in serum following injection of actidione, o-dichloroacetyloxine, prodigiosin, or stilbamidine isethionate (TABLE IV). The results with serum obtained following administration

TABLE III

MEDIAN EFFECTIVE DOSE VALUES FOR C. TROPICALIS WITH ANTIFUNGAL DRUGS IN 50 PERCENT NORMAL SERUM BROTH

Drug	MED in repeated experiments (mcg per ml)	MED mean (mcg per ml)
Actidione	0.09, 0.10, 0.16	0.12
Ascosin	0.46, 0.48, 0.58	0.51
Candicidin	5.6, 6.0, 6.2, 6.2	6.0
o-Dichloroacetyloxine	3.7, 3.9, 4.6	4.0
3-keto-21- (piperidyl)-Δ^4-pregnadiene monohydrobromide	0.10, 0.10, 0.10, 0.20	0.12
Nystatin	1.9, 1.9, 2.2, 2.4, 2.4	2.2
Prodigiosin	39, 42	40
Rimocidin	4.6, 4.7, 4.7	4.7
Stilbamidine Isethionate	4.6, 6.3, 6.5	5.8

of rimocidin (TABLE IV) suggested the presence of a trace too small to be accurately measured. It will be noted that when any of the sera giving negative assays were fortified with the corresponding drug, recovery of the added material was good, confirming the absence of endogenous activity for *C. tropicalis*. These observations do not rule out the existence of antifungal activity in the blood at some other interval following administration of these drugs, or its existence in some other fraction of the blood, or following higher dosage.

With the available sample of prodigiosin, the arbitrary intraperitoneal dose, 16 mgm per kg, represented only 0.4 MED per gm of body weight. As a measurable blood level could hardly be anticipated with this intraperitoneal dose, an additional assay of prodigiosin was performed, using blood from animals given 6 mgm per kg intravenously. The results of the assay indicated a prodigiosin serum concentration of 70 to 80 mcg

per milliliter. This approximates the anticipated level with this dosage. When actidione, o-dichloroacetyloxine, or stilbamidine isethionate were given intravenously, no measurable antifungal activity of the serum was found.

TABLE IV

ANTIFUNGAL BLOOD SERUM LEVELS FOLLOWING INTRAPERITONEAL INJECTION OF FUNGICIDES

(16 mgm per kg; blood collected 2.5 to 3.5 hours following injection of drug)

Serum obtained following injection of:	Actual mcg of drug per ml of serum			No. MED per ml serum	Recovery of added drug		
					mcg/ml of drug added to serum	Recovery	
	Assay 1	Assay 2	Average			Theoretical	Actual
Actidione	0	0	0	0	1.0	1.0	1.5
Ascosin	1.1	2.3	1.7	3.3	(1)3.0 (2)1.0	4.7 2.7	4.4 3.3
Candicidin	28.0	28.0	28.0	4.7	24.0	52.0	49.0
0-dichloracetyloxine	0	0	0	0	16.0	16.0	10.6–18.0
3-Keto-21-(piperidyl)-Δ^4-pregnadiene HBr	1.2	1.1	1.2	12.0	1.0	2.2	2.2
Nystatin (serum batch A) (serum batch B)	 20.4 28.6	 19.6 22.2	 20.0 25.4	 9.1 11.5	 10.0* —	 10.0 —	 10.0 —
Prodigiosin	0	0	0	0	160.0	160.0	140.0
Rimocidin (serum batch A) (serum batch B)	 1.5–2.0 0	 1.5–2.0 0	 1.5–2.0 0	 — 0	 1.0 24.0	 2.5–3.0 24.0	 2.5–3.0 24.0
Stilbamidine isethionate	0	0	0	0	150.0	150.0	166.0

* Insufficient test serum for recovery experiment. Recovery of drug added to normal serum is shown.

With ascosin, candicinin, 3-keto-21-(piperidyl)-Δ^4-pregnadiene monohydrobromide, and nystatin substantial levels ranging from 3.3 to 12.0 MED per milliliter of serum were found. Recovery of measured amounts of drug added to the test sera corresponded reasonably well with the theoretical expectancies.

Representative assay data for some of the drugs are given in TABLE V.

TABLE V

ANTIFUNGAL ACTIVITY OF BLOOD SERUM, FOLLOWING INTRAPERITONEAL INJECTION OF FUNGICIDES

(16 mgm per kg; blood collected 2.5 to 3.5 hours following injection of drug)

Dose-response of *C. tropicalis* in 50% normal serum broth		Dose-response of *C. tropicalis* with test sera	
Drug mcg/ml	Numbers of blastospores per unit volume	Serum dilution	Numbers of blastospores per unit volume
A. Ascosin			
0.00	1000	0.1	962
0.40	601	0.2	861
0.60	383	0.3	719
0.80	118	0.4	688
1.00	33	0.5	395
		Assay: 1.2 mcg/ml	
B. Candicidin			
0.00	1000	0.1	1018
3.00	754	0.2	638
4.00	693	0.3	341
5.00	658	0.4	171
6.00	556	0.5	70
7.00	337		
8.00	269		
10.00	59		
		Assay: 28 mcg/ml	
C. 3-Keto-21-(piperidyl)-Δ^4-pregnadiene monohydrobromide			
0.00	1000	0.025	854
0.05	973	0.050	752
0.07	765	0.100	584
0.10	520	0.200	568
0.20	333	0.300	419
0.30	225		
0.40	134		
0.50	83		
		Assay: 1.1 mcg/ml	
D. Nystatin			
0.00	1000	0.05	940
1.00	815	0.10	625
2.00	644	0.20	232
2.40	586		
3.20	427		
4.00	161		
		Assay: 22.2 mcg/ml	

DISCUSSION

A preliminary examination has been made of some possibilities for the specific, quantitative detection of fungicides in biological materials by means of microbiological assay, utilizing a pathogenic fungus as the indicator organism. Detailed procedures for the routine estimation of particular fungicides were not attempted. It is obvious that each drug will present separate, novel, minor problems on full investigation; and that microbiological procedures may be inapplicable in some instances, as when the action of a drug is indirectly fungicidal through enhancement of the host's protective mechanisms. It is believed, however, that the techniques outlined above will find numerous, valuable applications in the laboratory investigation and evaluation of antifungal drugs for therapeutic use. No serious problems were encountered with nine different drugs, indicating the method's versatility. Results were reproducible and recovery of added known amounts of fungicide was good.

Several sera from animals which had received certain antibacterial antibiotics and steroid hormones markedly accelerated the growth of *C. tropicalis*. This presented the chief obstacle to satisfactory assay, but did not interfere to a significant extent with the recovery from such sera of added antifungal drug. Sera with this peculiar growth-accelerating property are under further study in this laboratory. It is possible that there may be some correlation with the activation of fungous disease which is sometimes seen when certain antibacterial antibiotics and steroid hormones are administered.

SUMMARY

Applying as a standard the growth of *Candida tropicalis* (Castellani) Berkhout in 50 percent normal guinea pig serum containing dilutions of fungicide, the antifungal activity of guinea pig serum obtained after intraperitoneal injection of various antifungal drugs was measured. Results were reproducible within satisfactory limits and recovery from the test sera of added known amounts of fungicide was good. While no *in vivo* levels were detectable for actidione, o-dichloroacetyloxine, prodigiosin, or stilbamidine isethionate under the imposed experimental conditions (dose of 16 mgm per kg regardless of biological potency of drug; blood taken approximately three hours post injection), substantial serum concentrations ranging from 1.2 to 28 mcg per ml were found for ascosin, candicidin, 3-keto-21-piperidyl)-Δ^4-pregnadiene monohydrobromide, nystatin, and rimocidin.

It is proposed that further development of such microbiological technique may furnish a valuable tool in the laboratory investigation and evaluation of antifungal drugs for therapeutic use.

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INORGANIC NUTRITION OF MYROTHECIUM VERRUCARIA

RICHARD T. DARBY AND G. R. MANDELS

(WITH 4 FIGURES)

The composition of inorganic salt solutions used for the cultivation of most fungi has generally been obtained empirically, it being early recognized that while variations in the formulae would affect growth, the effects would probably be of minor significance for the intended purpose of the experiment. The present paper reports experiments concerning growth of *Myrothecium verrucaria* on several substrates in solutions of systematically varied composition. This imperfect fungus was unknown in experimental microbiology until recently. It has high cellulolytic activity, rapid growth and simple nutritional requirements; the organism sporulates readily, the spores occurring in moist masses. These characteristics have led to its wide use in experimental microbiology and as a test organism in studies of microbiological degradation. Taxonomic studies of the organism have been made by White and Downing, **1947**. Studies of its cellulolytic activity have been carried out by Greathouse, **1942, 1950**; Saunders *et al.*, **1948**; Siu and Sinden, **1951**; Reese *et al.*, **1952**; Blum and Stahl, **1952**; and Whitaker, **1953**. It has been used to develop an accelerated test for the determination of susceptibility of various materials to microbiological degradation (Mandels and Siu, **1950**) and for the rapid screening of compounds for fungitoxicity (Mandels and Darby, **1953**). The respiratory metabolism of its mycelium has been studied by Darby and Goddard, **1950**. Several papers have appeared characterizing the carbohydrate metabolism and surface location of certain enzymes in its spores (Mandels, **1953a**, **1954**). Additional studies with its spores include a general treatment of respiration and germination (Mandels and Norton, **1948**), and descriptions of an atypical surface-located ascorbic acid oxidase (Mandels, **1953b**). Shirk and Byrne (**1951**) have used its spores in a respirometric assay of a large series of nitrophenols. The production of antibiotics by this and related species has been noted by Brian *et al.* (**1948**). Nearly all of the 70 papers in which studies on this organism have been reported have appeared within the last decade. The references cited in this brief review are not

complete. The reader is referred to the papers listed for additional references.

METHODS

Stock cultures of *M. verrucaria* (PQD *460* = USDA *1334.2*) were carried on potato dextrose agar or on filter paper on salt agar. Spore suspensions for inocula were prepared by flooding the surface of stock cultures with distilled water and shaking gently. The suspensions were not washed. Unless stated otherwise, the inorganic nutrient solution hereafter referred to as basic salts having a pH of 6.4 contained NH_4NO_3—3.0 g (37.5 mM); KH_2PO_4—2.68 g (19.7 mM); K_2HPO_4—2.09 g (12 mM); $MgSO_4 \cdot 7H_2O$—2.22 g (9 mM); 0.1 g Difco yeast extract; dist. H_2O—1 liter. Two percent sucrose or ground cellulose (40 mesh, 12 oz gray cotton duck) was used as a carbon source. CP grade chemicals were used. At least three replicates were used for each treatment.

TABLE I

EFFECT OF SHAKING AND RATIO OF FLUID VOLUME TO FLASK SIZE ON GROWTH
(Basic salts, 2% sucrose, 0.01% yeast extract)

Condition	Vol. of sol'n	Flask size	Dry weight of mycelium per ml		
			2 days mg	5 days mg	28 days mg
Still	25 ml	125 ml	1.1	4.0	2.5
Shaken	25 ml	125 ml	3.4	5.8	3.0
Shaken	25 ml	250 ml	5.6	5.1	2.4
Shaken	50 ml	125 ml	2.3	4.8	2.9
Shaken	50 ml	250 ml	3.7	5.7	3.1

Erlenmeyer flasks received 25 ml/125 ml or 50 ml/250 ml of medium containing *ca.* 10^6–10^7 spores/ml and were incubated at 30° C on a reciprocal shaker having 90 3″ strokes per minute. These conditions were selected as a result of preliminary work shown in TABLE I.

Requirements for trace elements are apparently satisfied by impurities in the reagents used and in the yeast extract. Addition of these metals collectively at the concentrations indicated below was slightly inhibitory to growth on sucrose. Dry weights at 2, 5 and 7 days were 40, 99, 109 without, and 25, 63, 98 ml/25 ml with trace elements. Trace elements were added as the following salts (mg/l): $Fe_2(SO_4)_3$ hydrate, 54; $(NH_4)_3P(Mo_3O_{10})_4$, 24; $ZnSO_4 \cdot 7H_2O$, 50; $CuSO_4 \cdot 5H_2O$, 2.5; $MnSO_4 \cdot H_2O$, 5.5.

Agar cultures (1.5% Difco agar) contained *ca.* 40 ml/10 cm petri dish. Linear growth was determined as the mean of two diameters

each of three replicate plates and is expressed as radial increase in mm/day.

Dry weights were obtained by collecting and washing the mycelium on filter paper discs and drying overnight at 80° C. An average tare weight was automatically subtracted by use of a counter-weight with a Roller-Smith torsion balance. This was justified by the following analyses:[1] (a) 16 mm diameter discs, sharkskin filter paper (cut by a special hand punch), $X = 8.58$ mg, $s = 0.185$ mg, $n = 116$; (b) 40 mm discs (Schleicher and Schuell No. 589–1H), $X = 76.83$ mg, $s = 2.482$ mg, $n = 294$. Thus in 95% of the cases ($\pm 2s$) the maximum error introduced was 0.37 and 4.96 mg for (a) and (b) respectively. For net weights of 10 and 100 mg the paper error is less than 5%. Since most weights exceeded these values, the error was correspondingly less.

RESULTS

(1) *Effect of modification of nutrient salt solution on growth in solution*

To determine the effect of wide variations of the inorganic salt concentrations on growth with sucrose as carbon source, the basic salt solution was varied by 5-fold steps. Each of the 3 salts was used at three levels, making 27 solutions in all, plus a no-salts control. Solution F–2 (TABLE II) approximates the basic salts solution. The phosphate was changed to contain equal molarities of KH_2PO_4-K_2HPO_4, pH 6.8. Dry weights and final pH at 2, 4 and 7 days are shown in TABLE II. Best results were obtained with solution B–3 containing $NH_4NO_3 = 7.5$ mM, $K_xH_xPO_4 = 160$ mM, $MgSO_4 = 1.8$ mM.

In a further experiment the relative concentration of the salts of the B–3 solution was maintained, but the total salt concentration was varied from 5 × to 1/500 (TABLE III). Most rapid initial growth occurred in the 5 × solution, although greatest total growth in 5–7 days was obtained with the 2 × solution. Growth falls off with decreasing total salts until at the lower levels it approaches that of the no-salts control. At this point the effects of the carry-over of unknown quantities of salts with the unwashed inoculum and that contained in the yeast extract become complicating. Differences in results between similar solutions in TABLES II and III are ascribed to normal variation.

[1] $\bar{X}$ = arithmetic mean, $\frac{\text{sum of individual weights}}{\text{number}}$

s = standard deviation = $\sqrt{\frac{\Sigma x^2}{n}}$

where x = deviation of the individual values from the mean

n = number of determinations.

TABLE II

EFFECT OF VARIED CONCENTRATIONS OF SALTS ON MYCELIAL WEIGHT AND pH (2% sucrose, 0.01% yeast extract)

	Time	Concentration		Dry weight per culture (mg/25 ml)			Final pH		
				Concentration $K_2H_2PO_4$ (mM)					
		NH_4NO_3	$MgSO_4$	6.4	32.0	160	6.4	32	160
	days	mM	mM	1	2	3	1	2	3
A	2	7.5	0.36	27	25	34	5.8	6.4	6.6
	4			55	52	85	6.6	6.7	6.9
	7			59	60	85	7.5	6.6	6.6
B	2	7.5	1.8	30	23	45	6.0	6.5	6.8
	4			72	64	84	6.1	7.1	7.3
	7			94	95	142	6.6	6.8	6.6
C	2	7.5	9.0	36	25	39	6.0	6.4	6.7
	4			65	64	98	6.2	6.9	7.0
	7			83	78	127	6.2	6.7	6.8
D	2	37.5	0.36	28	22	43	5.8	6.4	6.6
	4			53	51	54	7.0	7.1	7.1
	7			56	57	74	7.0	7.1	6.7
E	2	37.5	1.8	28	23	44	5.8	6.0	6.5
	4			60	75	95	6.1	6.3	7.1
	7			70	78	105	6.5	6.5	6.8
F	2	37.5	9.0	28	38	35	6.4	6.2	6.6
	4			84	68	94	6.4	6.7	6.9
	7			64	46	120	6.8	6.9	6.5
G	2	187.5	0.36	23	25	32	5.9	5.9	6.9
	4			41	45	47	6.9	6.1	7.1
	7			46	41	57	6.5	6.9	6.7
H	2	187.5	1.8	38	23	34	5.6	5.9	6.4
	4			60	69	74	6.2	7.1	7.0
	7			58	68	101	6.8	6.7	6.6
I	2	187.5	9.0	22	26	24	5.7	6.0	6.5
	4			33	48	66	6.0	6.7	6.8
	7			52	71	89	6.4	6.5	6.7
No salts (control)	2				11			5.6	
	4				26			5.5	
	7				27			4.7	

Further data on the total salt effect can be seen by comparing A1, E2 and I3; A2 and E3; B1 and F2; B2 and F3; D1 and H2; D2 and H3; and E1 and I2 in TABLE II. In almost every case better growth occurs at the higher concentrations.

TABLE III

EFFECT OF TOTAL SALT CONCENTRATION ON DRY WEIGHT
(2% sucrose, 0.01% yeast extract)

Concentration of salts (mM)				Dry weight per culture (mg/25 ml)			
Expt.	NH_4NO_3	$K_2H_2PO_4$	$MgSO_4$	Rel. conc'n*	2d	5d	7d
1	37.5	800	9.0	5	158	142	160
1	15.0	320	3.6	2	130	172	158
2	15.0	320	3.6	2	36	161	203
2	7.5	160	1.8	1	28	112	160
2	1.5	32	0.36	1/5	21	76	108
2	0.15	3.2	0.036	1/50	32	54	86
2	0.015	0.32	0.0036	1/500	30	73	35

* In comparison with solution B-3 in TABLE I.

Varying the total nitrogen concentration over a wide range results in strikingly different types of growth curves (FIG. 1). At the same level of sucrose (0.1M) the NH_4NO_3 concentration was varied from 1.5 to 500 mM, the other salts being the same as in the basic solution. The initial rate of growth—i.e. for the first two days—is a direct func-

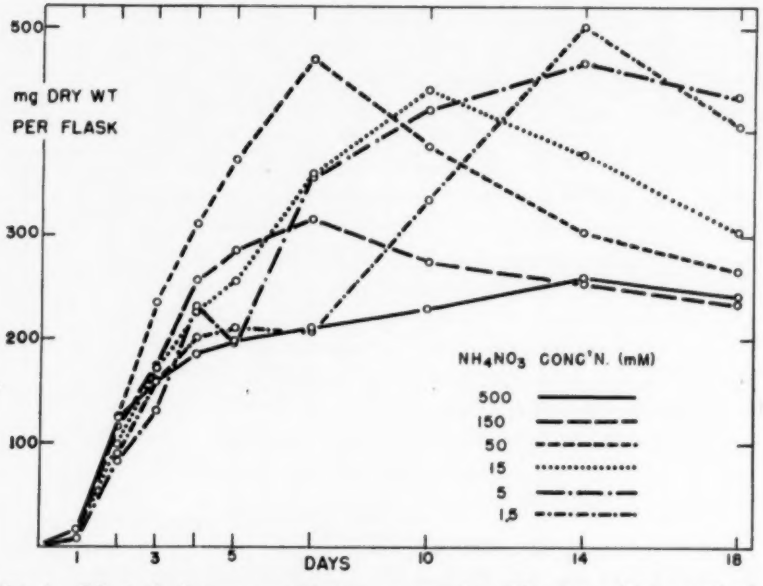

FIG. 1. Effect of nitrogen concentration on growth. (Mg. dry weight per 50 ml. basic salts + 0.1 M sucrose (3.4%) + NH_4NO_3 as indicated, 30°.)

tion of the nitrogen concentration. On the other hand it is surprising to note that the maximum amount of mycelium produced tends to be an inverse function of the amount of nitrogen. More unusual are the double peaked curves for the two lowest levels of nitrogen—one early, followed by a short period of autolysis, then a resurgence of growth to a second high peak. This suggests a shift in metabolism, perhaps due to utilization of excreted organic nitrogen compounds. Only minor pH

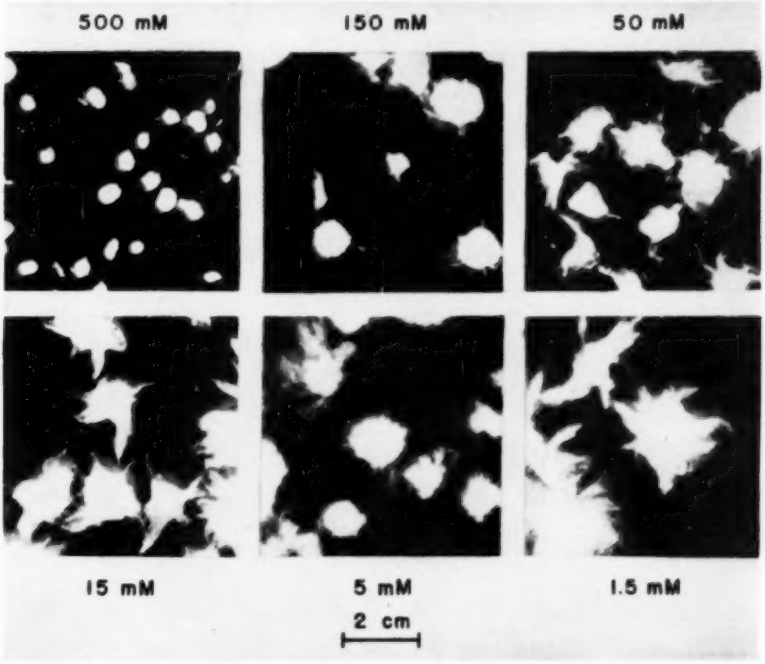

FIG. 2. Types of pellets formed with different concentrations of NH_4NO_3. (32 mM $K_2P_xPO_4 + 9$ mM $MgSO_4 + 0.1$ M sucrose (3.4%), NH_4NO_3 as indicated, 30°, 18 days.)

changes occur during growth, the extremes being about 6.0 and 7.5, and these do not coincide with the breaks in the growth curves.

Differences in the character of growth in shaker flasks at nitrogen concentrations are very pronounced (FIG. 2). At high levels the pellets are small, compact and smooth. As the nitrogen is decreased the pellets become larger, more diffuse, with a more hairy type of growth. Also,

the pale yellow pigment which is characteristically produced with most media is much reduced at the lower nitrogen levels.

Observations of the size and shape of pellets in other experiments were not recorded, although more or less comparable variations have been observed.

TABLE IV

EFFECT OF SALT CONCENTRATION ON CELLULOSE BREAKDOWN

I. Loss in dry weight of cellulose-mycelium mixture
(500 mg ground cellulose/25 ml, 0.01% yeast extract added, 13 days growth)

	NH_4NO_3 (mM)	$MgSO_4$ (mM)	1	2	3
			$K_2H_2PO_4$ (mM)		
			3.2	32	320
			%	%	%
A	7.5	0	—	—	19
B	7.5	0.9	17	13	10
C	7.5	9.0	38	12	27
D	75	0.9	17	24	21
E	75	9.0	17	47	23
F	750	0.9	41	46	31
G	750	9.0	47	37	16

No salts control = 10% loss in dry weight

II. Loss in breaking strength of cotton duck
(1"×3" strips on salt agar, 0.01% yeast extract added, 13 days growth)

			%	%	%
A	7.5	0	—	—	92
B	7.5	0.9	56	—	42
C	7.5	9.0	94	54	43
D	75	0.9	83	82	74
E	75	9.0	78	100	68
F	750	0.9	93	98	92
G	750	9.0	98	97	93

No salts control = 38% loss in breaking strength.

(2) Effect of modification of nutrient salts solution on cellulose breakdown

Modification of the salts solution appears to have somewhat different effects when cellulose is the substrate (TABLE IV). Optimum growth, as measured by loss in total dry weight of the cellulose-mycelium mixture, is noted in solutions E–2, F–1,2 and G–1,2. Compared to growth on sucrose, the requirements for phosphate are considerably less, while high nitrogen is more beneficial.

The breaking strength data in this particular experiment are of limited value as measures of relative rates of cellulose breakdown because the prolonged incubation could have permitted complete loss in strength in solutions where breakdown was actually relatively slow. In general the results are comparable to those noted above. The high activity in the absence of added $MgSO_4$, which has been noted previously (Siu and

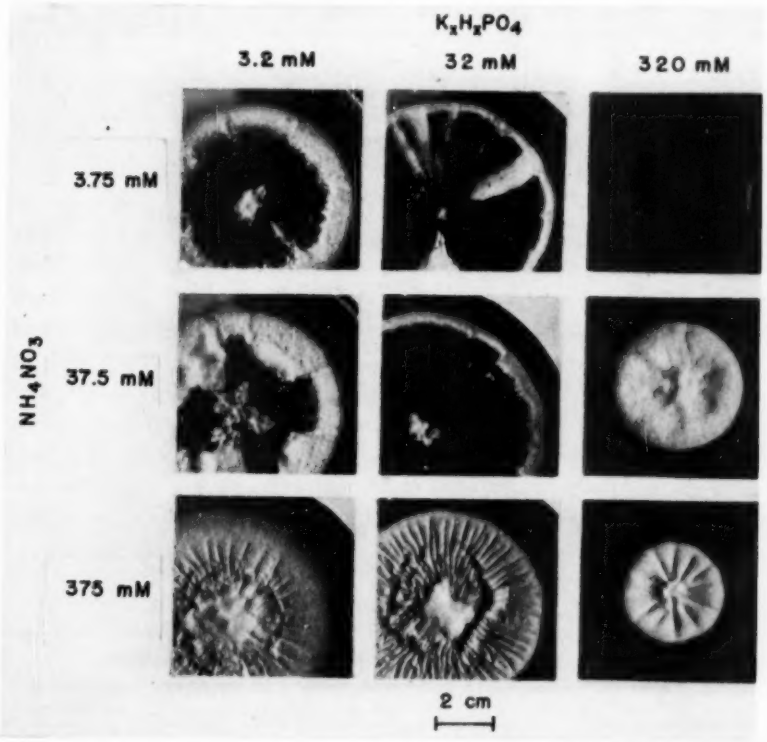

FIG. 3. Effect of inorganic composition of the medium on growth on agar + 2% sucrose, 0.01% yeast extract, and 9 mM $MgSO_4$. 15 days. Initial pH 6.8.

Sinden, **1951**), indicates a low requirement which is met by impurities in the cellulose.

(3) *Effect of modification of nutrient salts solution on growth on agar*

The effects of modification of the basic salts solution on growth on agar containing sucrose are quite different from those in liquid culture (FIG.

TABLE V

GROWTH ON VARIOUS INORGANIC NITROGEN SOURCES
(2% sucrose + 0.01% yeast extract)

	Dry weight per culture (mg/25 ml)				pH		
	Days	NH_4Cl	KNO_3	NH_4NO_3	NH_4Cl	KNO_3	NH_4NO_3
Expt. 1	2	28	25	36	2.8	6.2	6.2
	4	40	39	62	2.7	6.2	7.2
	7–8	53	96	94	2.6	7.0	7.2
Expt. 2	3	47	19	45			
	6	113	117	112			
	10	135	150	125	6.4	6.4	6.4

Expt. 1 $K_xH_xPO_4$ = 32 mM, $MgSO_4$ = 9 mM.
Expt. 2 $K_xH_xPO_4$ = 320 mM, $MgSO_4$ = 1 mM.
Total nitrogen in all flasks was 105 mM.

3). Since varying magnesium sulfate from 0.09–9 mM had no effect on the rate or character of growth, only the results at 0.9 mM are illustrated. High potassium phosphate markedly suppresses linear growth and sporulation, while at low concentrations the thickness of growth and amount of sporulation are slightly decreased. High concentrations of nitrogen suppress sporulation. Sectoring is occasionally encountered in this organism. In the plates shown in FIG. 2 frequent sectoring is noted where sporulation has occurred. The apparent absence of sectors where no sporulation occurred is of interest. Sparse growth at the normal linear rate occurs on sucrose-agar to which no salts have been added.

TABLE VI

UTILIZATION OF ORGANIC NITROGEN

N source*	Dry weight per culture (mg/25 ml)		
	3d	6d	10d
Sodium monoglutamate	78	182	157
dl-Isoleucine	35	177	150
dl-Aspartic acid	63	155	148
dl-Alanine	45	128	166
Glycine	30	101	150
l(+)Arginine monohydrochloride	12	74	111
Asparagine	34	150	173
Urea	28	98	138
NH_4NO_3 (control)	45	112	125

Total N in all flasks = 20 mM, $K_xH_xPO_4$ = 320 mM, $MgSO_4$ = 10 mM, pH 6.4, 2% sucrose + 0.01% yeast extract.
* Autoclaved separately.

(4) *Nitrogen nutrition*

In shaker flasks with sucrose as substrate, no great differences in growth occur with nitrogen supplied as NH_4^+, NO_3^- or as both, except in poorly buffered media containing NH_4^+ where the pH drops rapidly and growth is poor (TABLE V). On agar, with either cellulose or glucose as substrate, the character of growth varies considerably with these sources of nitrogen (FIG. 4). Effects of pH changes or their magnitude are not known here.

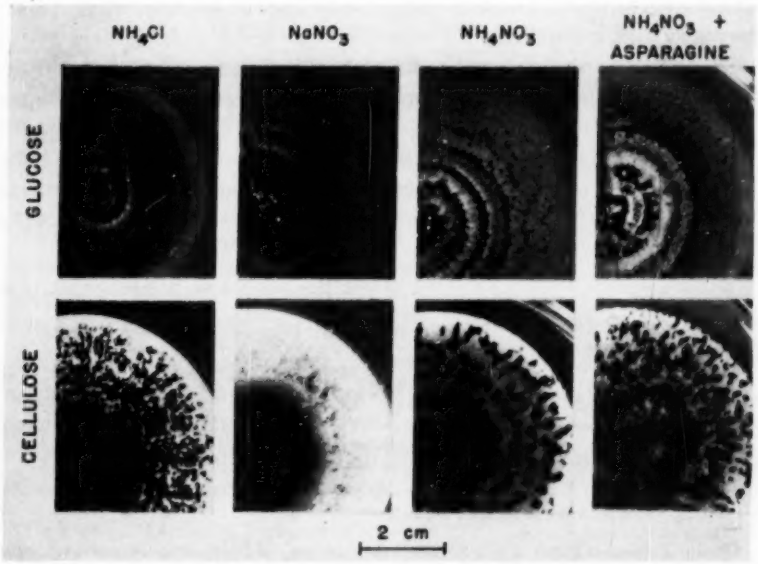

FIG. 4. Effect of inorganic nitrogen source on character of growth with glucose and cellulose as substrates. Basic salts plus 0.5% glucose or Whatman No. 2 filter paper. Nitrogen supplied as a) NH_4Cl, b) $NaNO_3$, c) NH_4NO_3, d) $NH_4NO_3 + 0.1\%$ asparagine. 17 days old.

Growth with various sources of organic N are shown in TABLE VI. Sodium monoglutamate and isoleucine were superior to the other amino acids tested. Alanine, asparagine and aspartic acid were excellent, somewhat better than the several inorganic forms of nitrogen tried. Glycine was about as good as inorganic N; arginine was somewhat inferior to inorganic N. A mixture of amino acids as found in casein hydrolysate was excellent, as shown in other experiments (not reported).

DISCUSSION

Growth occurs over wide modifications of the inorganic nutrient solution. Varying total salts by a factor of 1000, the ratios of the salts by factors of 25, or nitrogen by 300, affects the initial rate of growth on sucrose, for example, only by a factor of about 2 and total growth about 3.5 times. Variations from one experiment to another, using the same nutrient solution, are as great or greater than those induced experimentally. The type of growth is influenced greatly by modification of the inorganic nutrients. This is illustrated strikingly for growth on agar where sporulation is affected qualitatively and in solution using pellet morphology as a criterion.

The selection of a culture medium should, therefore, be governed by the end results desired. Media may thus be selected for ease of prepa-

TABLE VII

RECOMMENDED CONCENTRATIONS OF SALTS FOR VARIOUS TYPES OF GROWTH

Criterion	Substrate	Composition (grams/liter)[1]			
		NH_4NO_3	KH_2PO_4	K_2HPO_4	$MgSO_4 \cdot 7H_2O$
Dry weight in solution	sucrose	1.2	21.8	27.9	0.9
Mycelium + spores on agar	sucrose	0.3	2.2	2.8	0.2
Mycelium on agar	sucrose	3.0	2.2	2.8	0.2
Dry weight loss	ground cloth	6.0	2.2	2.8	0.2
Tensile strength loss	cloth strips	6.0	2.2	2.8	0.2
Tensile strength loss[2]	cloth strips	0.3	0.7	0.9	0.05
Cx production[3]	ground cellulose	1.0	1.36	—	0.3
Spore production[4]	filter paper	3.0	2.7	2.1	2.2

[1] 0.01% yeast extract is usually added.
[2] Data of Siu and Sinden.
[3] Data of Reese (**1950**), Cx a cellulolytic enzyme, pH adjusted to 6.3 with N/1 NaOH.
[4] Mandels and Norton, **1948**.

ration, maximum dry weight in solution, maximum growth on agar, cellulose breakdown, production of pigment, enzyme or other chemicals, spore production or suppression, pH control, etc.

A summary of approximate salt concentrations found to be most favorable for various types of growth and metabolic activity is presented in TABLE VII. While the conditions under which these results were obtained are not strictly comparable, the data represent an approach to the formulation of optimum solutions.

The favorable effect on dry weight of relatively high phosphate and low nitrogen and magnesium sulfate cannot be explained on its high

buffering capacity since the organism has never been observed to produce much acid, and it is tolerant of a wide pH range. On the basis of the data of Siu and Sinden (**1951**) its superiority might be expected to be due to the high phosphate rather than to potassium. Unpublished data support this idea.

The salt requirements for optimum cellulose breakdown vary with the criteria used and are widely different from those required for growth on sucrose. When based on loss in total dry weight of the cellulose-mycelium mixture the advantages of the high nitrogen solutions are evident, whereas high phosphate is not beneficial. When based on loss in tensile strength of cotton duck the addition of very low concentrations of salts is enough to give almost maximal breakdown. It is, in fact, possible to get very high breakdown without added magnesium sulfate, and the no salts control suffered 38% loss. Siu and Sinden (**1951**) found 0.6 mM potassium optimal for cellulose breakdown, although about 50% loss occurred without added potassium. Salts contained in the cloth represent an unknown factor.

SUMMARY

Optimum concentrations of inorganic salts for *M. verrucaria* depend upon whether the experimental requirements are for dry matter production, pellet size and shape, vegetative *vs.* reproductive growth on agar, cellulose breakdown, etc. Good growth occurs with either NO_3^- or NH_4^+, provided the pH is controlled, although better growth (with sucrose) occurs with organic nitrogen.

PIONEERING RESEARCH LABORATORIES,
U. S. ARMY QUARTERMASTER CORPS,
PHILADELPHIA 45, PENNSYLVANIA

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ANTAGONISTIC ACTIVITY OF CHAETOMIUM GLOBOSUM AGAINST FUNGI

JEAN CUMMINGS

(WITH 3 FIGURES)

In 1944, Waksman and Bugie (**3**) reported that several species of *Chaetomium* produced an antibiotic substance, chaetomin, active against gram-positive bacteria but inactive against fungi. *Chaetomium globosum* Kunze was listed as one of the species which did not form this substance. Karel and Roach (**2**) and Brian (**1**) reported chaetomin as the only antibiotic substance yet found in this genus. The present report is of a new type of antagonism within this genus.

MATERIALS AND METHODS

C. globosum, strain *6205*, was obtained from the American Type Culture Collection and single spore isolations were made. The progeny of one of these isolates was kept as a stock culture and was grown on filter paper strips on corn meal agar slants. All of the fungi tested, with the exception of three genera, *Pullularia* which was a laboratory stock culture, *Penicillium* which was found as a contaminant, and *Neurospora tetrasperma* ATCC *9457*, came from Dr. Norman Conant's laboratory, Duke University. These included: *Nocardia mexicanus, Nocardia asteroides, Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporum gypseum, Microsporum canis, Microsporum audouini* (this culture has been kept in stock for five years and is probably pleomorphic), *Blastomyces dermatitidis, Monosporium apiospermum, Histoplasma capsulatum, Sporotrichum schenkii, Phialophora verrucosa, Cryptococcus neoformans, Candida albicans, Candida krusei, Rhodotorula* sp., and *Verticillium* sp.

C. globosum was inoculated on filter paper strips on corn meal extract agar plates and incubated at room temperature for two weeks. The test organisms were than streaked across the agar to the filter paper and incubated at room temperature for 7 days. All tests were made at least in duplicate and control plates, made and incubated in exactly the same way but with no *Chaetomium* on the filter paper, were also inoculated with the test organisms.

RESULTS

Incubation at room temperature gave good growth of all organisms in the control plates within seven days, except for *B. dermatitidis* and *H. capsulatum*, neither of which grew well on the plates.

The results are given in TABLE I and indicate that, on plate cultures, all the fungi tested, with the exception of the unknown species of *Penicillium*, are susceptible to the antagonistic activity of *C. globosum*.

FIGS. 1–3 illustrate this activity for some of the test organisms. Partial inhibition shows plainly with *M. audouini* and *C. albicans*. The very

TABLE I

GROWTH OF TEST ORGANISMS ON CONTROL AND TEST PLATES

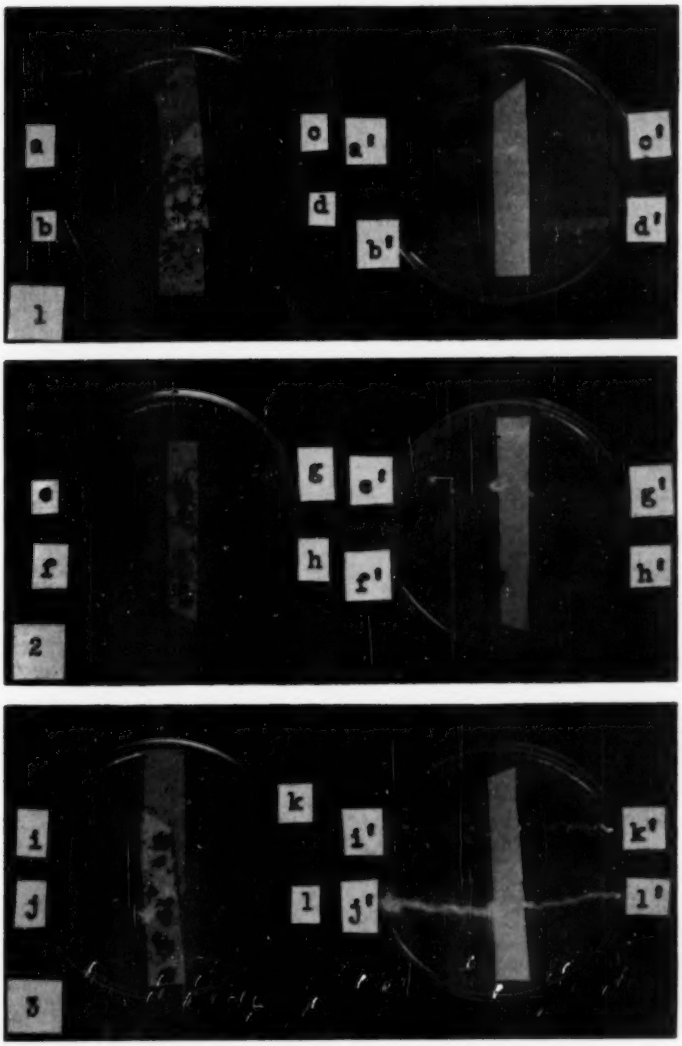
Organism	Control plate	Test plate
Cryptococcus neoformans	4	0 to 1
Candida albicans	4	0
Candida krusei	4	0 to 2
Nocardia asteroides	4	0
Nocardia mexicanus	1 to 2	0
Rhodotorula sp.	4	2
Trichophyton mentagrophytes	4	0
Trichophyton tonsurans	4	0
Microsporum gypseum	4	0
Microsporum canis	1 to 2	0
Microsporum audouini	4	sl to 1
Blastomyces dermatitidis	sl to 1	sl
Monosporium apiospermum	4	0 to 1
Histoplasma capsulatum	1 to 2	0 to sl
Phialophora verrucosa	4	sl to 2
Sporotrichum schenkii	4	0
Pullularia sp.	4	2
Verticillium sp.	4	1
Penicillium sp.	4	4
Neurospora tetrasperma	4	0

0 represents no growth; sl represents a trace of growth; 1 through 4 increasing amounts of growth. Two figures in one column indicate the variation in growth on different plates.

slight growth of *H. capsulatum* and *B. dermatitidis* preclude making any decisions in regard to these forms in the test plates. A test of *H. capsulatum* in test tube cultures, however, showed definite inhibition of this fungus.

DISCUSSION

The results show a clear-cut antagonistic activity of *C. globosum* against a number of fungi. When tested after two weeks growth, it completely inhibits half of the organisms tested and at least partly inhibits all the rest except one.



FIGS. 1–3. Test plates with *C. globosum* and control plates without *C. globosum* streaked with the organisms indicated. 1. a,a′ *C. albicans;* b,b′ *Cry. neoformans;* c,c′ *C. krusei;* d,d′ *N. asteroides.* 2. e,e′ *H. capsulatum;* f,f′ *S. schenkii;* g,g′ *B. dermatitidis;* h,h′ *M. apiospermum.* 3. i,i′ *M. gypseum;* j,j′ *M. audouini;* k,k′ *T. mentagrophytes;* l,l′ *T. tonsurans.*

The strength of the antagonism in these tests is a function of the age of the *C. globosum* culture. When tested after six days of growth, only partial inhibition of susceptible tests organisms occurs. It is difficult to assess the activity after more than two weeks of growth on the plate because of the widespread growth of perithecia of *C. globosum* over all the surface of the plate. This makes comparison of the amount of growth of the test organisms much more difficult.

Further work to determine whether other species and strains of *Chaetomium* exhibit the same antagonism is being carried out. Attempts are also being made to determine whether a diffusible antibiotic substance is present or whether the inhibition of the test organisms is due to exhaustion of some essential growth factor.

DEPARTMENT OF BIOLOGY,
WESTERN RESERVE UNIVERSITY,
CLEVELAND 6, OHIO

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HOST REACTION, HOST–PARASITE RELATIONSHIP, HOSTS, AND TAXONOMIC CRITERIA IN SYNCHYTRIUM

JOHN S. KARLING

Although all species of *Synchytrium* are parasitic, the majority of them are not very destructive to the host plant. When heavily infected, the leaves, stems and fruits of the host may become malformed, distorted and discolored, as in cases of infection by *S. vaccinii, S. sesamicola, S. oxalidis, S. geranii, S. cookii,* etc., but ordinarily the host as a whole is not seriously affected. *Synchytrium endobioticum,* on the other hand, may be a very destructive parasite, and when it infects very young tissues of susceptible potato varieties gall and tumor development may be so pronounced that the affected organ or tissue bears but little resemblance to its normal form. In such extreme cases the organ involved may be but little more than an irregular mass of meristematic and poorly differentiated parenchymatous cells with a few interspersed vascular elements.

The other species, although not so destructive, nevertheless, produce distinct local effects on the host. These are due primarily to cell enlargement, or cell multiplication (meresis), or a combination of both. The infected cell is almost always stimulated to enlarge by the presence of the parasite, while the neighboring cells may or may not enlarge and divide. The result is the development of galls or warts of varying sizes and degrees of complexity. These galls are primarily histoid, according to Küster's (**1911**) terminology, in that they usually involve only certain tissues. On the other hand, as noted above, in instances of infection of very young organs, and particularly when the galls become confluent, marked abnormalities occur. However, such compound galls do not consist of more or less recognizable organs in the true sense of Küster's organoid galls.

Since in most species only epidermal and rarely subepidermal cells are infected, the galls are made up chiefly of slightly modified and undifferentiated cells, and in several species they consist of a single enlarged epidermal cell or hair, as in *S. papillatum, S. trichophilum, S. potentillae, S. amsinckeae,* and *S. myosotidis.* These represent the so-called simple galls of Fischer (**1892**). The galls of *S. papillatum* on *Erodium cicu-*

tarium which bear resting spores are particularly noteworthy in that they break away from the leaf epidermis and fall to the ground at maturity. This results from the development of a thin ring- or band-shaped area around the stalk of the gall at the level of the neighboring epidermal cells, and when the resting spores are mature the gall breaks off at this ring. By such means the resting spores are eventually liberated on the damp ground beneath the host where conditions are favorable for their germination.

In the majority of species, however, the galls are composite in that they are multicellular and composed of healthy as well as infected cells. These also may vary markedly in size, shape and complexity. Furthermore, within a single species, i.e. *S. cellulare, S. modioliensis,* etc., the galls produced may vary strikingly on the basis of whether they contain sporangial sori or resting spores. A fairly common type of composite sporangial gall is illustrated by *S. mercurialis* which is somewhat globular and protruding, and consists of an enlarged infected cell surrounded except at the apex by one or more layers of adjacent epidermal cells. The latter cells in some species may become infected also, with the result that compound galls are produced. Sometimes the neighboring cells merely enlarge without dividing. Galls of this structure usually do not project conspicuously above the surface of the host, and if they become confluent like other composite galls they may form the so-called crust galls (Krustengallen) described by Rytz (**1907**). In other species, like *S. pilificum* and *S. stereospermi,* the adjacent epidermal cells are reported to elongate and become filamentous, so that the infected cell is surrounded by a tuft of hairs. Such galls correspond closely to the felt galls (Filzgallen) of Küster.

In *S. endobioticum,* the species which so far has been studied most intensively of all from the standpoint of host reaction, the galls produced vary markedly on the basis of whether they occur on the under- or above-ground parts of the host and are the result of infection by zoospores or zygotes. According to Köhler (**1925, 1928, 1931**) scattered infections of the above-ground organs of susceptible varieties by zoospores lead to the development of peculiarly constructed radiate (radiär) galls, a type of circumvallated (Umwallungsgallen) gall in which the almost superficial parasite becomes overgrown and enclosed by the growth of the host tissue. A raised ring tumour develops from the meristem around the site of infection from which leaf-like organs in a single or double whorl arise. If the prosorus of the fungus is ripe or its content has emerged and divided into sporangia, the infected cell, on the other hand, may be surrounded only by a rosette of epidermal cells. The radiate galls may

be stalked, raised, and protruding, sessile, or sunken (Köhler, **1925**). Highly raised and stalked galls occur exclusively on the stem and leaf petioles, according to Köhler. The stalked galls usually contain differentiated vascular elements which may extend up into the abortive leaf primordia and connect with the vascular tissue of the stem and petioles. On the underground organs, infection by zoospores causes massive circumvallated tissue development around the site of infection in which differentiation may be lacking or greatly reduced. As a result the leaf primordia undergo marked changes and finally become irregular, thickened, fleshy coralloid structures.

When infection by zygotes occurs the host tissue reacts differently. The infected epidermal cell as well as the adjacent ones are stimulated to divide whereby the incipient resting spore becomes progressively deeper buried in the host tissue. In this manner irregular, warty excrescences or tumors are formed which may become remarkably large in size. Bally (**1911**) designated these as well as all other galls produced by *S. endobioticum* as kataplasmatic galls because they consist largely of parenchyma and are only slightly, if at all, differentiated.

In several species like *S. oxalidis, S. vulcanicum, S. geranii, S. cookii,* etc., the galls produced frequently become compound, large, and confluent and may include vascular elements. In such galls the parasite and infected cell are reported to become buried in the tissues by the rapid multiplication of and development by adjacent cells. In other species, i.e. *S. dendriticum, S. indicum,* etc., however, the isolated galls are reported to be so small and inconspicuous that the infected areas appear as minute light- to dark- and reddish-brown flecks or spots.

Obviously, as noted before, the shape, size and structure of the galls produced may be quite variable. Whether or not they are specific and characteristic for individual species of *Synchytrium* and can be used effectively in identification and classification is open to question and has been frequently disputed. Following the precedence set by DeBary and Woronin (**1863**) and Woronin (**1868**) in establishing the genus, most subsequent workers have included a description of the galls in their diagnoses of species. However, except for a few species, these descriptions have not been of great benefit to investigators unfamiliar with individual species of *Synchytrium* because of the variations exhibited by the galls within a single species. In his summary of species known up to that time, Schroeter (**1870**) emphasized strongly that gall structure alone cannot be used in species identification because the type of gall produced is a reaction between host and parasite and not a species character.

Fischer (**1892**) appears to have been the first to use this character extensively as an adjunct in distinguishing the species in the subgenus *Pycnochytrium*, but apparently he did not believe it could be employed effectively in differentiating members of *Eusynchytrium*. In the subgroups *Leucochytrium* and *Chrysochytrium* under *Pycnochytrium* he described the galls or warts as simple (Simplica) and composite (Compositа) and used these types as prominent criteria in his key to the species. He defined the simple galls or warts as single, unicellular, and composed entirely of an enlarged or swollen epidermal cell. The composite warts were described as complex and multicellular, and consisting of an enlarged epidermal cell surrounded by a crateriform sheath, which is composed of one to several layers of neighboring epidermal cells. Under each of these types he made additional distinctions on the basis of whether the galls are hyaline or colored, bare and naked, hairy, stalked, low, high, punctiform, pearl-like, or if the surrounding sheath is thin and weakly developed or thick and massive. That Fischer regarded gall structure as highly significant for species identification is indicated by his comment (p. 55) on *S. cupulatum* relative to specimens distributed as *S. globosum* on *Potentilla reptans:* "The fungus distributed as *S. globosum* on *Potentilla reptans* definitely does not belong here because this form produces composite warts, which in itself constitutes a specific difference." In this particular case Fischer was obviously correct.

Ludi (**1901**), on the other hand, maintained that gall structure is not a reliable diagnostic character and has little value in the identification of species on the ground that a wide range of galls may be produced by the same species. He analyzed as an example the galls which had been described for eight species and noted that in each of them the galls varied from simple to composite with thin and partially developed, or thick surrounding sheaths. Ludi, nevertheless, believed (pp. 10, 11), "that under otherwise similar conditions the form of the wart, occurring on anatomically similar organs of the same plant species or of wholly different host plants, show a certain constance. . . . That, however, does not demonstrate that the similar form of wart is a specific characteristic of the fungus; it is more appropriate to regard it as a consequence of the morphological structure of the infected organ."

Rytz (**1907**) disagreed to some extent with Ludi's view that gall structure is of little significance and emphasized the necessity of ascertaining the characteristic gall structure of every *Synchytrium* species on every host plant. In an intensive study of *S. aureum* on a large number of out-of-door host plants he distinguished seven forms on the basis of pri-

mary hosts, size and shape of resting spores and sporangia, and the types of galls produced on the primary and secondary host plants. Rytz, thus, attached a great deal of significance to the nature of the galls in differentiating these forms. Nevertheless, he emphasized the necessity of distinguishing single and, what he called, crust galls (Krustenwarzen) as a basis of comparison. In his opinion, only the former offers an opportunity of finding the gall that is characteristic of the causative fungus. He pointed out further that the structure and complexity of the galls depend on additional factors such as organ or tissue infected, i.e., leaf, petiole or stem, length of growth period of organ or tissue infected, and whether infection occurred on young meristematic or mature and fully grown tissues. Therefore, in determining the characteristic galls produced by different species comparisons should be made only between single or solitary galls occurring on the same organs or tissues which have the same length of growth period and were infected at the same stage of maturity.

Minden (**1911**) revived Fischer's use of simple and composite galls as a criterion in his key to the identification of the species in *Pycnochytrium*, but he did not think it could be applied to species in *Eusynchytrium*. In using this criterion, however, he recognized its limited use, applied it in the sense of Rytz, and emphasized that only single galls should be considered. In her monograph of *Synchytrium* Tobler (**1912**) included a description of the galls with the diagnosis of species but pointed out that very seldom is gall structure specific.

This controversial issue has been revived in recent years by Cook (**1945–53**) who described and created a large number of new species primarily on the basis of gall structure. His views on the value of gall structure in identifications were stated in several papers as follows (**1945C**, p. 176): "These gall structures appear to be more characteristic and more satisfactory for determinations and descriptions than the characters of the fungi." and (**1953**, p. 101): "The shape and structure of the galls [is] more definite and valuable for descriptions and identification than any of the characters of the fungus." In the author's opinion, Cook's statements are not justifiable, particularly not for the species he created. From his meager descriptions it seems that the fungi were not studied intensively enough to determine their specific characteristics. In most species the descriptions were limited to one developmental phase of the life cycle, and frequently it is impossible to determine whether the described phase relates to sori, prosori, or resting spores. Furthermore, so little of their life cycle was described that it is not certain whether they are short- or long-cycle species. The lat-

ter is very significant in relation to gall structure because in a large number of long-cycle species the galls produced by the soral and prosoral phases may be quite different from those caused by the resting spores. In addition the illustrations presented do not always support his viewpoint. In the writer's opinion, some of the photographs of *S. mitchellae* galls (**1953**, fig. 41), for instance, are strikingly similar to those of *S. trachelospermi* (fig. 44). Also, the galls shown of *S. bignoniae* (fig. 29) are very similar to those of *S. callicarpae* (fig. 38). At least, the pictorial evidence presented is not very convincing. Instead, his identification of these as new species appears to be based more on host specificity than on gall structure.

In the short-cycle species, i.e. members of the subgenera *Woroninella* and *Pycnochytrium*, which produce only sporangial sori and resting spores respectively, there seems to be more uniformity in gall structure, and for such species this character may be more helpful in identification. Nevertheless, here also gall structure may vary in individual species, and it is essential that the predominant type and structure as well as its variations be carefully ascertained. However, in a few species of *Pycnochytrium* the resting spore galls appear to be quite distinctive and specific. In *S. pilificum*, for instance, the adjacent epidermal cells are reported to elongate and become filamentous, so that the infected cell is surrounded by a tuft of hairs. Also the galls of *S. potentillae*, *S. myosotidis*, and *S. punctatum* are reported to be specific. In the subgenera *Eusynchytrium* and *Mesochytrium*, which include only long-cycle species, the resting spore galls of some species are very distinctive, as noted previously. Also, in *S. trichophilum* both the sporangial and resting spore galls are simple and unicellular, according to Tobler (**1912**). In *S. steriospermi* also, both types of galls are similar and distinctive in that they are surrounded by stout, pointed hairs.

Outside of these species there are very few which have been reported to produce distinctive and specific galls. However, it should be emphasized here that very little intensive study on the development, types, and variations of galls has been made for most *Synchytrium* species, and at present it is impossible to say to what extent the galls are specific in the genus as a whole. Most descriptions and diagnoses of the long-cycle species do not specify which galls are being described, and it is impossible to determine whether they relate to sporangial or resting spore galls. Inasmuch as they usually vary markedly, it is highly essential that both types be studied and described separately.

From the reported data in the literature on *Synchytrium* concerning

the reaction of the infected as well as neighboring cells several categories may be enumerated, as Tobler (**1912**) and Rytz (**1916**) pointed out:

1. Enlargement confined to the infected cell; neighboring cells little or not at all affected: *S. pyriforme, S. trichophilum, S. papillatum, S. myosotidis, S. laetum, S. punctatum, S. rubrocinctum, S. anomalum, S. amsinckeae, S. borreriae, S. nyctanthidis,* etc. This reaction results in the development of simple, unicellular galls.

2. Neighboring, as well as infected cells, becoming more or less enlarged but not dividing: *S. anemones, S. aureum* var. *saxifragae,* etc.

3. Neighboring epidermal cells dividing repeatedly with or without much enlargement to form a single- to several-layered, protruding sheath partly or almost completely around the enlarged infected cell: *S. mercurialis, S. auranticum, S. holwayi, S. lepedii, S. modioliensis,* etc. This type of reaction is reported to be the most common so far as the species are known, and usually leads to the development of protruding composite galls.

4. Infected cell expanding or growing largely inward, partly compressing and probably inhibiting growth of the deeper-lying cell layers: *S. ulmariae* and *S. johansonii.* This reaction may occur occasionally in other species which normally form slightly or prominently protruding galls, as reported by Magnus (**1874**) for *S. rubrocinctum* and Ludi (**1901**) for *S. taraxaci.* It occurs occasionally in the case of the resting spore galls of *S. modioliensis* also.

5. Infected, as well as neighboring cells, dividing: *S. endobioticum.*

6. Infected cell enlarging only slightly or not at all, and soon dying: *S. fulgens* on *Oenothera sinuata.*

7. Infected and neighboring cells apparently unaffected by the presence of the parasite (Cartwright, **1926**), or becoming chronically or acutely necrotic and causing disintegration of the parasite (Köhler, **1928, 1931**): *S. endobioticum* on immune potato varieties.

8. Healthy neighboring cells swelling and arching or expanding inward on the dead infected cell and undergoing periclinal divisions whereby the host cell and sorus are ruptured, discharging the sporangia from the host: *S. endobioticum* on resistant potato varieties. According to Köhler, this discharge (Ausstössung) reaction is initiated by the release of a substance or necro-hormone from the injured or dead host cell which stimulates the neighboring cells to grow and divide with walls periclinal to the focus of injury or infection. A corky abstriction layer or secondary periderm is thereby formed and the parasite is extruded. This leads to a self-cleansing and healing of the tissues.

9. Infected cells becoming elongate and locally enlarged without dividing; neighboring cells apparently unaffected: *S. zygogonii, S. longispinosus,* etc., in algae.

10. Walls of infected and compressed adjacent cells dissolved by the action of the parasite, forming a large lysigenous cavity in the host tissue; parasite surrounded by a symplast: *S. minutum* and *S. aecidioides.*

The last-named reaction was described by Kusano (**1907, 1909**) for *S. minutum.* The infected cell rarely increases to more than twice its normal diameter, but space necessary for the unusually large sorus is provided in another manner. Even before the parasite has reached the limits of the host cell it apparently secretes an enzyme which dissolves the wall of the infected as well as those of adjacent cells. Thus, a large lysigenous cavity is formed. Before the sorus reaches maturity, however, the ability to dissolve walls is lost. As it increases further in diameter the unaffected neighboring cells become flattened into a multicellular layer around the parasite. In the meantime, the protoplasm of the cells whose walls have been dissolved fuse to form a living, healthy, multinucleate symplast around the sorus. Further enlargement of the parasite reduces the symplast to a very thin layer, but it is not absorbed or consumed. According to Kusano, its gradual disappearance is due to self-disorganization. This interpretation, however, does not appear to be supported fully by his illustrations. In the early stages of infection the host nuclei appear normal, but with the growth of the parasite they become greatly enlarged and distorted. In the final stages they become flattened and disc-shaped against the sorus. As the sorus matures and segments into incipient sporangia, the adjacent cells elongate into the lysigenous cavity and thus apparently push out the powdery mass of sporangia. Kusano described the same type of reaction for *S. aecidioides,* and subsequently Bally (**1911**) reported it in *S. taraxaci.*

Cells of *Oenothera sinuata* do not enlarge to any marked degree when infected with *S. fulgens.* According to Kusano (**1929**), this is due to the relative resistance of the host. The reaction is probably necrogenious and aborting. The cells and the parasite die shortly after infection, and their nucleus and cytoplasm degenerate simultaneously with those of the parasite. The cells of *O. odorata,* on the other hand, are susceptible and favorable to the development of the parasite, but the presence of a thick epidermal wall is a defense barrier to its entry. Hence, this species is seldom found to be infected in the field.

Synchytrium endobioticum is the only species of the genus so far reported which stimulates the infected cell to divide, and in this respect it is said to be similar to *Plasmodiophora brassicae, Sorosphaera veronicae,*

and other members of the Plasmodiophoraceae. According to Bally (**1911**), Curtis (**1921**) and Köhler (**1928, 1931**), this reaction depends, however, on whether the infected cell is parasitized by a zoospore or zygote. In the former instance the cell only enlarges and eventually dies, while in the latter it divides repeatedly. When infected by both zoospores and zygotes the influence of the former predominates, according to Curtis, and the host cell enlarges more than usual.

Other reactions of the host to *S. endobioticum* vary markedly and depend on the relative susceptibility and immunity of its host. In weakly susceptible varieties of potatoes the reaction is not pronounced. Instead of typical cauliflower-like excrescences relative insignificant circular swellings are produced. In highly susceptible varieties, on the other hand, extensive malformations of buds and shoots occur, as noted previously, resulting in the characteristic galls and warty growths in tubers, stolons and the base of the leaf stalks. In these varieties the parasite and host, nevertheless, appear to tolerate each other in the sense that the parasite is not killed or eliminated. According to Gäumann (**1950**), this tolerance is comparable to that existing between the bacteria and host in root nodules of leguminous plants and represents a case of eusymbiosis.

In resistant potato varieties the reaction is quite different. The host and parasite are incompatible, according to Gäumann (**1950**), and they react antagonistically to one another. The zoospores of *S. endobioticum* infect the epidermal cells quite readily, according to Cartwright (**1926**), and develop normally for a few days. Subsequently, however, no trace of the parasite can be found, and Cartwright concluded that it degenerates without killing or otherwise affecting the infected and adjacent cells. Köhler (**1928, 1931**), on the other hand, reported that the infected cell becomes necrotic. By using young potato sprouts, he found that resistant and susceptible varieties are equally-well infected with zoospores and zygotes. In the resistant varieties, i.e. Ackersegen, however, the parasite dies within a few hours or days because of the death of the host cell. The protoplasm of the latter is converted into a strongly chromogenic alveolar mass, and as a result the infected cell loses its turgidity and is strongly compressed by the neighboring cells. Not only is the parasite injured by the necrotic products, but it is deprived of nourishment by the death of the host protoplast.

Köhler described this degeneration of the parasite as necrotic abortion, and recognized two types: acute and chronic. In the former, as described above, the infected cell dies within a few hours or days after infection. In the latter, which is exemplified by the reaction of certain

immune varieties like Prussian, the neighboring cells are the first to become necrotic, while the infected cell at first remains unaltered. Apparently, certain deleterious substances diffuse from the parasitized cells which produce a general gummosis and death of the surrounding cells and thus isolate or delimit the infected cell. As a result the latter as well as the parasite die within 2 to 3 weeks. In other varieties, i.e. Industry, the whole necrotic region, together with the parasite, is delimited or cut out by a zone of thin-walled meristematic cells and degenerates.

Resistance in the immune varieties, according to Cartwright (**1926**), is not due to mechanical barriers like a thick epidermis, etc., but to a physiological condition in the host cell, which is unsuitable for the development of the parasite. Köhler, however, found that varietal resistance is dependent on three closely related factors: (1) number of epidermal layers present, (2) reaction potential of the variety, and (3) the relative ability of the parasite to spread downward and cause subinfection.

All known aquatic species which were formerly included in *Micromyces* cause marked local hypertrophy of the infected algal cells, but do not stimulate them or the adjacent ones to divide. In addition, the infected cells may become two to three times longer than healthy ones, because the presence of the parasites prevents nuclear and cell division but apparently does not inhibit cell elongation. Also, the longitudinal and transverse walls of the infected cells may become thickened. In the local area occupied by the parasite a marked increase in diameter and bulging out of the host cells occurs so that this region may become almost conical and two to three times the normal diameter of the cell. According to Huber-Pestalozzi (**1931**), the plastids and the rest of the protoplasm become plasmolysed and clumped shortly after the parasite enters, but Heidt (**1937**) failed to find this immediate reaction. Nevertheless, the plastids change to light-yellow or yellowish-brown in color, and as degeneration continues the residual protoplasm becomes aggregated in the enlarged region near or around the parasite. The wall of the swollen region or conical protuberance eventually bursts, and most of the residue may escape to the outside through a large cleft or pore.

The effect of the parasite on the host nucleus is often very extensive. In *S. anemones, S. endobioticum, S. zygogonii, S. longispinosus,* etc., the newly-entered parasite takes up a position near the host nucleus, and in these species at least there seems to be a distinct attraction between the two. Orton and Kern (**1919**) reported that *S. endobioticum* engulfs the host nucleus, which may be visible in the resting spore un-

til after the zoospores are formed. Their account has not been confirmed, and in light of subsequent studies it is not improbable that what they believed to be the host nucleus is the residue of the primary nucleus of the resting spore. In the aquatic species, the incipient prosorus migrates almost at once to the host nucleus and appears to develop at its expense. As a result the host nucleus is soon reduced to a small, densely stainable body on the surface of the prosorus. Apparently, no enlargement of the host nucleus occurs as the result of infection by these species.

In *S. anemones, S. mercurialis, S. anomalum, S. endobioticum,* and probably in other species, on the other hand, the host nucleus usually becomes greatly enlarged, irregular, and gradually disintegrates, according to Lowenthal (**1905**), Guttenberg (**1909**), Percival (**1910**), Bally (**1911**), Tobler (**1912**) and others. In the first three species mentioned above, the nuclei may attain diameters of more than 50 μ. They usually become very irregular in outline and extensively ramified by furrows and canals. Such changes are accompanied by a marked enlargement of the nucleole and its subsequent fragmentation into a large number of secondary nucleoli which then enlarge and become intensely basiphilic. Similar but less marked changes may occur also in the nuclei of neighboring cells.

Some effects of the parasite on the host cytoplasm have been noted above in relation to the aquatic species, and mention has been made of the extensive necrosis and gummosis in infected and neighboring cells of immune varieties of potatoes infected by *S. endobioticum.* In the majority of species, however, little is known about the effects on the host cytoplasm. In the case of *S. anemones,* the infected cell becomes densely filled with coarsely alveolar cytoplasm, while the adjacent cells become almost empty. Similar but less extensive increases in the amount of the protoplasm of the infected cell have been reported for plants parasitized by *S. minutum, S. anomalum,* and in *S. mercurialis,* but in the case of the last-named species the adjacent enlarged cells likewise become richer in cytoplasm and plastids. As the infected cells enlarge more and more, the cytoplasm becomes increasingly vacuolate and forms a thin layer around the parasite. After the latter has reached maturity the residual cytoplasm, degenerated plastids and starch grains usually persist as a brownish, crumbly, dry or gummy and homogeneous crust around the sorus.

In cells infected by *S. viride* and *S. pyriforme* a marked increase in chlorophyll content occurs, whereby they become densely green. In cases of infection by *S. auranticum, S. rugulosum, S. rubrocinctum, S.*

geranii, etc., anthocyan (Ludwig, **1890**) is formed in the infected and neighboring cells, which imparts a characteristic color to the galls.

The changes induced in the cell wall vary considerably. As noted above in the cases of *S. minutum*, *S. aecidioides* and *S. taraxaci* the walls of the infected and adjacent cells are reported to be dissolved by the action of the parasite, while *S. mercurialis*, *S. anemones*, *S. globosum*, *S. succisae*, *S. aureum* and the aquatic species noted previously may cause marked thickening and punctation of the walls. Such changes may often extend to the walls of the adjacent cells as well. In the majority of species, however, very little is known about the changes which they induce in the cell walls.

With the exception of those relating to *S. endobioticum*, *S. fulgens* and *S. minutum*, many of the host reactions reported in the literature have not been studied intensively, and it is quite possible that several of the reports may prove to be incorrect or inadequate when the host reactions and developmental stages of the galls have been investigated closely. At present this appears to be true of the accounts of some of the species noted in category 3 in which the neighboring epidermal host cells are reported to divide repeatedly with or without enlargement to form a single- to several-layered sheath partly or almost completely around the infected cell. In *S. modioliensis*, at least, the writer has found that, contrary to Cook's (**1945C**) account, the sporangial galls arise by repeated division of the infected cell and its derivatives, and that the adjacent healthy epidermal cells take little, if any, part in gall development. The discovery that the infected cell in this species divides, also shows that *S. endobioticum* is not the only species which stimulates the infected cell to divide. Intensive studies on gall development of other species, i.e. *S. oxalidis*, *S. geranii*, *S. cookii*, etc., may reveal similar evidence, and in view of this it is obviously essential that the host reactions of the individual species be studied intensively from early infection to mature stages.

Although the subgenera may be recognized and determined by the type of life cycle and development, species identification in most cases is more difficult because many of the species do not differ markedly. Consequently, in addition to differences in life cycle and development other criteria such as size, shape and color of the sori, prosori, resting spores, sporangia and zoospores; number, position and orientation of sori and resting spores in the host cell; structure and color of the resting spore wall; presence, amount and appearance, or absence of the protoplasmic residue around the resting spore; size of sori, prosori and resting spores relative to that of infected cell; differences in cytology; en-

vironment; structure, appearance and color of the galls produced, host range, and specialization have been used as adjuncts to identify species. However, as Ludi (**1901**) pointed out more than fifty years ago, these criteria may be so variable that in many instances they have but little significant diagnostic value.

Size, shape and structure of the sori, prosori, resting spores, sporangia and zoospores ordinarily are the most reliable characters, but species determination and comparison on this basis involves a full knowledge of the successive developmental stages of the species. This usually necessitates intensive and often prolonged study of individual species, which most taxonomists have not had the time or been willing to do. Also, the sori, prosori, resting spores and sporangia may vary so markedly in size in individual species that their ranges of variation overlap those of other species. This was emphasized by Ludi (**1901**) who showed that the range of variation in eight species was almost the same. Further evidence of this can be found by comparison of the numerous species which have been discovered since that time. Furthermore, the size of sori, prosori and resting spores may depend on the number present in a single host cell. Likewise, the number is not always constant and specific, and may vary from one to twenty in a species. Similarly, the shape may vary from almost spherical, globular and angular to elongate, depending on the number present and the fundamental shape of the cells of an infected organ. In *S. anomalum, S. lateum, S. globosum, S. alpinum,* etc., the resting spores may be distinctly angular and polygonal when several are present in a host cell. Also, the resting spores of *S. lateum* in the elongate leaf rib cells of *Gagea lutea* may be correspondingly elongate, while those present in the shorter cells may be globular or almost spherical.

Color has been used extensively in classification, and most species diagnoses include a description of the color of the contents and wall of the prosori, sori, resting spores and sporangia whenever these successive developmental stages are known. Schroeter (**1870**) used this character as a primary criterion in establishing the subgenera *Eusynchytrium, Chrysochytrium* and *Leucochytrium,* but as the latter two subgenera were made sub-groups of *Pycnochytrium* color began to be used largely in relation to the content and wall of the resting spore. However, as Ludi (**1901**), Cook (**1953**) and others have indicated, color is not always a reliable specific character and does not appear to be as significant taxonomically as it was believed to be. The resting spore content of the majority of species is reported to be very similar or almost identical in appearance. Furthermore, the color may vary greatly with the degree

of maturity of the sori and prosori as well as the resting spores. Some species may be almost hyaline when young and gradually become darker yellow to deep orange with maturity. Therefore, to be reliable, distinctions on the basis of color must be made on mature stages of the species. Also, it is not improbable that aging or prolonged storage in herbaria influence color of the resting spore contents.

Likewise, the color of the resting spore wall does not appear to be very reliable in diagnosis. It has been described as hyaline, dark, dull or gleaming in various species, but its appearance is probably influenced to some extent by age also. Furthermore, it is often difficult to determine the exact color of the wall in species where the spore content is pigmented or when the spore is surrounded by a layer of protoplasmic residue, which obviously influence the image in transmitted light. To be accurate, determinations of wall color should be made on free and isolated resting spores without content.

The structure of the sorus, prosorus or resting spore wall, on the other hand, is more reliable and can be used to identify some species. In most species the wall is smooth, but in others it may be sculptured, ridged, warty, or spiny. In *Microsynchytrium* all but one species have spiny prosori and resting spores, and in this subgenus distinctions are made on the relative length, number and appearance of the spines. Nevertheless, even here the same species occasionally develops smooth as well as spiny prosori. Additional examples of distinctive wall structure may be cited: in *S. endobioticum* the outer resting spore wall is chitinous, furrowed, ridged, or irregularly thickened; in *S. niesselii*, and *S. punctatum* it is warty, rugose, or echinulate; in *S. mercurialis*, it may be smooth, stippled, and transversely or spirally ridged; in *S. selaginellae* it bears minute, short spines, and in *S. rugulosum* it may be finely crimped or wrinkled. However, as noted above, it is smooth in most of the species and offers no clues to identity.

In several species it was noted quite early that the resting spores in particular were surrounded by and embedded in a layer of crusty brown protoplasmic residue, while in others the residue was sparse or lacking. Accordingly, the presence, amount and appearance, or absence of such residual material began to be used as an adjunct in differentiating species. In *S. aureum* (sen. strict.), *S. globosum*, *S. anemones*, *S. rytzii*, *S. myosotidis*, *S. incrassans*, *S. wurthii* and *S. australe*, for example, it is reported to be abundant and to some degree characteristic, but in most of the remaining species it may be sparse or absent. Also, in some species such as *S. lateum*, *S. anomalum*, etc., it is usually but not always present. In *S. aureum* (sen. strict.) it is very abundant, but in forms *infestans*,

alpicola and *vulgatum* of this species very little residue is present, according to Rytz (**1907**). Therefore, in view of these reported variations, this does not appear to be a very specific characteristic, and its value in species identification is limited and questionable. The amount of residue present is obviously the result of host reaction, and quite probably it will be found to vary in the same species when grown on different host plants.

Also, the size of the parasite relative to the infected cell is a doubtful and questionable character for diagnostic purposes. Nevertheless, whether or not the parasite fills the host cell is used to some extent in species descriptions. However, as Cook (**1945–53**) has reported for several species, the infected cell may enlarge more rapidly than the parasite with the result that it is only partly filled. Later, the parasite enlarges more rapidly so that the size relations between the two become quite different. Therefore, in some species at least the respective sizes of infected cell and parasite depend on degrees of relative maturity, and observations which are made before maturity may be inaccurate in this respect. Likewise the position and orientation of the parasite in the host cell are questionable characters. In some species the prosorus has been reported to lie in the upper (*S. holwayi, S. stellariae*) or the lower part of the infected cell so that the sori of sporangia are formed below and above the prosorus, respectively. In these two species, at least, the position of the parasite appears to be characteristic. In other species the long axis of the resting spore is reported to lie transverse, parallel or diagonal to that of the host cell. However, except for a very few species there does not appear to be any constancy in this respect, and these differences in orientation do not seem to be of much value in identification.

In *S. endobioticum* differences in cytology have been used to some extent as the basis for differentiating it from other species of *Synchytrium* and for excluding it from this genus. Percival (**1910**), Bally (**1911**), Curtis (**1921**) and Köhler (**1923**) maintained that the primary nucleus of the resting spore does not divide just prior to germination but periodically discharges chromatin or chromidia into the cytoplasm. Following the final discharge the nucleus shrinks and eventually disintegrates. The extruded chromatic granules grow in size in the cytoplasm and become the primordia or centers of zoospore development. The zoospores are not formed by cleavage of the protoplasm into uninucleate segments as in other species, according to these workers, but develop around and under the influence of the discharged chromatin granules, which become the definitive nuclei of the zoospores. Such differences in zoospore development have been regarded as distinctive, and Schilberszky (**1930**)

in particular used them together with other differences for placing *S. endobioticum* in a separate genus. Such reported differences appear to be due to incomplete and faulty observations, and further study of this species will probably show that its method of nuclear division and sporogenesis in the resting spore are similar to those of other species.

Differences in environment in which the fungi occur has been used also as an aid in differentiating some species and forms. All known members of the subgenus *Microsynchytrium* are wholly aquatic and parasitize algae, but this is not to be regarded as the most distinctive characteristic of the subgenus. It is not improbable that the type of development characteristic of *Microsynchytrium* will be found in terrestrial species also. Among the various forms of *S. aureum* which Rytz (**1907**) recognized, several of them were found to occur under different environmental conditions, and Rytz used such differences to some extent in distinguishing them. Forms *infestans, galli* and *drabae* were found only in a very moist environment, while forms *alpicola* and *vulgatum* occurred in arid places. However, the resting spores of all of them germinated in snow water (Schmelzwasser). In form *saxifragae,* on the other hand, they germinated only in flowing water, while in *S. aureum* sen. strict. the resting spores germinated only in still or standing water. Form *alpicola* is indistinguishable morphologically from *infestans,* according to Rytz, but because of its arid type of environment and different hosts he regarded it as distinct. Other species like *S. gei* and to some extent *S. potentillae,* appear to be alpine in habitat and are reported to occur in abundance on mountains up to 11,000 feet high, whose peaks are covered with snow until late spring and summer. The zoospores of *S. potentillae* have been found swimming in melted snow pockets. In contrast to these reports, the author has found this species at almost sea level in Virginia. Except for very few species, the special environmental conditions in which they occur are unknown, and this criterion may prove to be of very doubtful value. At least it can be used only to a limited degree at present.

Host range and host specificity are other criteria which have been used for identification of *Synchytrium* species. Ever since the genus was established there has been a tendency to create new species for each host infected, and this has been particularly evident in the past decade, during which more than 50 new species were created. This tendency is reflected in the reports that among the described species of *Synchytrium* 102 have been reported on only one host. So far there have been relatively few intensive studies on host range and specificity in the genus. However, many of the early as well as recent investigators who studied living spe-

cies in the field noted that one host species might be heavily infected while different species in close proximity were wholly free of infection, indicating thus some degree of host specificity. The first cross inoculation tests appear to have been made by DeBary and Woronin (**1863**, p. 47) who found that *S. taraxaci* could not be transferred to *Succia pratensis*. Schroeter (**1870**, p. 43) likewise inoculated *Lysimachia nummularia* and *Taraxacum* with *S. succisae* but failed to secure infection. Also, in 1889 Thomas showed by cross inoculation tests that *S. alpinum* will not infect *Adoxa moschatellina*, one of the hosts of *S. anomalum*.

However, it was not until the turn of the century, that any intensive controlled host range experiments were attempted. In 1901 and 1902 Ludi tested *S. taraxacum* on 11 genera and 19 species of non-Cichoraceae hosts and 9 genera and 21 species of the Cichoraceae and failed to secure infection except on species of *Taraxacum*. In this genus only seven species and five varieties proved to be susceptible. Thus, his studies demonstrated that *S. taraxacum* is limited in host range to a few species of *Taraxacum* and cast serious doubt on the reports of other workers on its occurrence on *Carduus, Crepis*, and *Achryophorus* species. McMurphy (**1913**) tested the zoospores of *S. amsinckeae* on *Erodium cicutarium* and those of *S. papillatum* on *Amsinckia intermedia* in the laboratory but failed to get cross infection. These results confirmed his observations in the field that neither *A. intermedia* nor *E. cicutarium* were infected by any but their respective parasites when growing closely together.

Gäumann (**1927**) likewise found that *S. psophocarpi* is limited to *Psophocarpus tetragonobolus* and will not infect *Vigna sinensis* and *Phaseolus lunatus*. Similarly, he discovered that *S. atylosiae* is confined to species of *Atylosia* as hosts and does not infect *Desmodium dependens, Vigna sinensis*, and *Phaseolus* sp. In *S. fulgens* Kusano (**1929**) reported that it occurs abundantly in Japan in *Oenothera lamarkiana* and *O. biennis* and will infect *O. odorata* and *O. sinuata*. However, on *O. odorata*, growing out-of-doors, the parasite is unable to penetrate the thick epidermis, while in the case of *O. sinuata* it dies shortly after entry. He did not make extensive tests with other species of *Oenothera*, and it is quite probable that *S. fulgens* attacks many species in the family *Onagraceae*, as other workers have reported.

Due to its economic importance *S. endobioticum* has been tested more intensively and extensively for its host range than any other species of *Synchytrium*. So far it has been reported by numerous workers to occur on several species and numerous varieties in six genera of the family Solanaceae. Nevertheless, it appears to be comparatively limited in host

range and will not parasitize most wild and cultivated solanaceous plants. Three virulent biological races of this species which will attack varieties of potatoes immune to ordinary strains of *S. endobioticum* were reported by Braun (**1942**) and by Blattny (**1942**) in Germany and Czechoslovakia.

Synchytrium aureum and its numerous forms or varieties, on the other hand, has been reported to occur on 186 species and 110 genera in 33 families of flowering plants. From careful studies in the field involving a large number of infected plants in nature Rytz (**1907**) found that numerous forms could be recognized by the fact that they have a primary host on which they occur most abundantly and secondary hosts which they infect sporadically. These conclusions were substantiated to a limited extent by his inoculation experiments (**1932**) which showed that the form with *Brunella vulgaris* as its primary host is different from the one occurring most abundantly on *Lysimachia nummularia*. More recently, the writer has found that *S. modioliensis* from *Modiola caroliniana* may be transferred to several malvaceous hosts.

In the remaining 140 or more described species no attempts have been made to determine their host range. Many which are morphologically similar have been described as distinct species because of their occurrence on different hosts. Most species in the subgenus *Woroninella*, for instance, are fairly similar morphologically and all occur only on species of the Leguminosiae. Possibly some of these may prove to be biological races when their host range is studied intensively. Studies of this nature on all species of *Synchytrium* will doubtless go a long way in determining the validity of many questionable species.

It would appear from the above historical resumé of the differences in reports and viewpoints on host reactions and the relative value of various taxonomic criteria that exact identification and classification of *Synchytrium* species is very difficult and, in some cases, almost impossible. Apparently, this is not so, and except for species which are very similar, overlap, or vary markedly in certain morphological characteristics, the difficulties seem to be due chiefly to lack of knowledge. As stated earlier by the author (**1953**), of the 140 or more described species less than 30 are fully known, and the identity of most of the remaining species is based on incomplete observations and meager descriptions. When these species become fully known the serious difficulties of classification and identity will probably disappear, as is evident from the history of other species. Obviously, more intensive and prolonged studies of individual species are needed, and if they are to be of much value they should include:

1. A complete investigation of the life cycle and successive developmental stages of the species;

2. A comprehensive account of the morphological characteristics of the species and their range of variation, i.e., size, shape, structure and color of the sori or prosori, sporangia, zoospores and resting spores;

3. Extensive inoculation experiments to determine the host range and specificity of the species;

4. Intensive anatomical and cytological investigations of the host reaction, i.e. size, shape, structure and appearance of the galls and infected cell, etc.

5. Critical observations of other differences such as habitat, conditions essential to development and resting spore germination, etc., which may be characteristic for the species.

Such studies, obviously, involve careful and intensive research over long periods of time, research which most of the recent investigators have not had the inclination or time to do. Of the criteria listed above, the first two are the most important, in the writer's opinion, because they relate directly to the fungi. If such studies fail to reveal specific morphological and developmental differences and characteristics, studies on host range, host reaction, and environmental conditions may show physiological differences which may be significant.

DEPARTMENT OF BIOLOGICAL SCIENCES,
PURDUE UNIVERSITY,
LAFAYETTE, INDIANA

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SOIL MICROFUNGI IN RELATION TO THE HARDWOOD FOREST CONTINUUM IN SOUTHERN WISCONSIN[1]

H. D. Tresner, M. P. Backus and J. T. Curtis

(with 6 figures)

Our store of information concerning fungi in the soil has grown steadily since the pioneer studies by Adametz in 1886, but the attention paid to ecological aspects of soil mycology has been relatively limited. The present study is concerned with the soil microfungi as community members of the upland forests of a well defined natural area. Conventional microbiological techniques, combined with some of the common measurements used by ecologists in studying higher vegetation, have been used in an attempt to determine the relationships existing between the microorganisms and the higher plants.

A series of upland hardwood forests within the prairie-forest border province of southern Wisconsin were selected as sites for the present soil-microbiological study. Curtis and McIntosh (**1**), in a study of the tree, shrub, and herb composition in 95 stands in this region, characterized the forests and also the physiography, climate, and soils. In their study, four tree species—*Quercus velutina, Q. alba, Q. rubra* and *Acer saccharum*—were found to be the leading dominants of the region based upon importance values which were calculated as the sum of the relative density, relative frequency and relative dominance. These four species, as well as other species with which they were found to be associated, were arranged in a series with *Quercus macrocarpa* as the most pioneer species and *Acer saccharum* as the most climax. The optimum development of each species occurred at some point along this series. No groups of species regularly occurred together; therefore no discrete communities were recognized.

By a summation procedure which involved weighting the measured importance values of each tree species by an index of their relative shade tolerance, it was possible to arrive at a single "continuum index" which indicated the position of any given stand along a gradient varying from

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a pioneer forest of *Quercus macrocarpa* with an index of 300 to a climax forest of *Acer saccharum* with an index of 3000. When such indices were calculated for all stands studied and the stands arranged in the numerical order given by the indices, it was possible to arrange a variety of independently derived information along the gradient and thus study its relation to the forest trees.

For example, characteristic patterns were found to exist in connection with the herbaceous plants and shrubs. Here, as with the trees, many of the species had minimum, optimum and maximum values for development along the continuum. However, a few ubiquitous forms were found to depart from this pattern, in that they covered the whole range of the continuum.

Of primary interest to the present microbiological study was the manner in which certain soil factors were correlated with the continuum. When the exchangeable calcium, the pH, the water retaining capacity and the organic matter content were plotted along the gradient of the continuum they increased from the pioneer to the more mesic or climax portions.

The amount of undecomposed leaf litter which accumulates is another important variable in the soil environment of the forests of this region. In the more pioneer stands of the forest continuum, accumulation of leaf litter is excessive, while in the more mesic or climax stands the amount is reduced. The soil profile itself tends to take on marked differences in stands along the gradient of the continuum, so that the soil environments at the two extremes are quite dissimilar, particularly at the surface or within the upper horizons. Since it has been quite generally established that most of the microbiological activity in the soil is carried on in the uppermost layers, it is here that major attention was focused in the present study.

Thirteen stands were studied, spaced at more or less regular intervals along the forest continuum and with index numbers ranging from 747 to 2680. These stands were situated on well drained upland soils; they had been undisturbed by excessive cutting, burning, grazing or cultivation. Each of the forests studied had previously been analyzed with respect to the higher vegetation, soil type and soil properties by Curtis and McIntosh (1).

The soils on most of the stands were sampled only once, during the summer months. Two stands from opposite extremities of the continuum were studied in somewhat more detail. Repeated samplings of these two stands were made in a study of the soil microorganisms during all seasons of the year. In addition, exploratory studies were made of

the microorganisms in the different horizons of the soil profile. The system used in designating the various layers of the soil profile was that used by Wilde (**10**), in which A_0 is raw humus or leaf litter, A_1 the incorporated humus layer, A_2 the eluvial or leached layer, B the illuvial or enriched layer, and C_1 the mantle rock or weathered parent material.

MATERIALS AND METHODS

Soil Sampling Methods

Soil samples were collected at regular intervals along a transect through a representative part of each tract selected for study. When sampling the A_1 horizon, a pit about six inches in diameter was dug which extended through this layer. A steel spatula, sterilized with 70% ethyl alcohol, was used to scrape the vertical wall of the pit to remove any contaminating soil present from other horizons. In obtaining a sample, a sterile glass vial, 20 × 80 mm, was forced horizontally into the uppermost part of the A_1 horizon to a depth adequate to assure a ten-gram sample for analysis. The vial was then withdrawn and stoppered. In this manner, soil for microbiological study was removed at each of six points along the transect line whenever a particular tract was sampled.

In preparation for sampling the various horizons of a soil profile, a trench about 1 × 3 feet was dug to the desired depth. With due precautions being taken to clean the vertical wall of the trench, samples were obtained from each desired level, beginning at the bottom. Soil was removed at approximately inch intervals in the upper portions of the profile, but at more widely spaced intervals in the lower strata.

Whenever soil samples were collected, duplicate samples were taken for the determination of soil moisture so that the number of microorganisms per gram of soil could be expressed on a dry weight basis. Soil samples were ordinarily processed immediately upon returning to the laboratory, but when this was not possible, the samples were refrigerated at about 5° C until analyzed. When high summer temperatures prevailed, the samples were transported to the laboratory in a pre-cooled thermos jug to prevent abnormal fluctuations in numbers of microorganisms.

Isolation Methods and Analytical Determinations

Since relatively large numbers of samples were to be processed and since it was desired to examine the fungal populations in some detail but the accompanying actinomycete and bacterial populations only super-

ficially, the simplest methods appropriate for obtaining the desired information in each case were adopted.

In connection with all the microbial analyses the dilution plate method was employed; sterile lake water was used to make the dilutions. In preparing the initial suspension of each soil sample, a 90 ml sterile water blank in an Erlenmeyer flask was weighed and while the flask remained in place on a balance 10 gms of soil were measured into it. The liquid in the flask was then thoroughly agitated to break up the soil particles. Further dilutions were made immediately and the ultimate suspensions were plated out as promptly as possible.

As far as bacteria and actinomycetes were concerned, the aim was simply to obtain an estimate of the total number of these organisms present; and for this purpose, in connection with all A_1 horizon samples, 1–500,000 and 1–1 million dilutions were used. One ml portions of the suspensions were added to sterile Petri dishes, three plates for each dilution plus a control with sterile water being prepared for each soil sample. About 15 ml of nutrient agar,[2] melted and cooled, were added to each plate and thoroughly mixed with the 1 ml portion of soil suspension previously introduced. The cultures, thus prepared, were incubated at room temperature. At the end of a week the total number of colonies developing in each plate was counted, and the plates were then incubated for an additional period of six or seven days. By this time the colonies of actinomycetes could be distinguished and their numbers determined. To obtain the number of bacterial colonies per plate, the number of colonies of actinomycetes was subtracted from the total.

A hard soil-extract agar was used as a substrate for the isolation and determination of numbers of soil microfungi. An extract of a sandy loam soil was prepared by heating 1 kilogram of the soil in 1 liter of tap water in an Arnold steam sterilizer for 1 hour at 100° C. The extract was filtered twice by suction filtration through a half-inch layer of diatomaceous earth covering an ordinary filter paper. Sterilization of the extract was accomplished by autoclaving it at 15 lbs pressure for an hour on each of two successive days. The soil extract medium had the following composition:

Agar	30.0 g
K_2HPO_4	0.5 g
$MgSO_4$	0.2 g
Glucose	0.5 g
Soil extract	100 ml
Distilled water	900 ml

[2] Per liter, agar 15 gms, Bacto-peptone (Difco) 5 gms, yeast extract (Difco) 3 gms, Cerelose (commercial hydrated glucose) 1 gm. The pH after sterilization was about 7.0.

The medium was autoclaved at 15 lbs pressure for 20 minutes. The pH was adjusted to about 4.5 by adding 85% lactic acid aseptically to the medium after it was melted for use. The isolation plates were prepared on a leveled table surface. Seven Petri dishes of the soil extract agar were poured for each soil sample and aligned on the table. After the agar had solidified, the position and orientation of the dishes were marked so that after being moved for subsequent treatment, they could be returned to their original location.

Except in profile studies, dilutions of 1–10,000 and 1–20,000 of each soil sample were used for fungal isolations. The plates were inoculated by adding one ml portions of these soil suspensions to the agar surfaces; three plates for each dilution, plus a control, were employed. After rotating the plates until the inoculum was distributed completely over the agar surface, they were returned to their original position on the table. After four days incubation of the seeded plates at room temperature, the number of fungal colonies developing on each plate was counted and the number of fungi per gram of dry soil was calculated.

Plates from one dilution of each soil sample, having from ten to thirty colonies each, were ordinarily selected for isolation of the organisms. Transfers were made from the colonies, beginning at one side of a Petri dish and taking them in order around the plate. Under a high-powered stereoscopic microscope a tiny fragment of agar bearing a few hyphal tips was removed from the margin of each colony with a sterile microscalpel and transferred to a tube slant of potato-dextrose agar. Thirty isolations were made from each soil sample.

After suitable incubation, the cultures were studied, sorted, and if possible identified. Naturally there were many cases in which species determinations could not be made. The difficulties here were those familiar to all mycologists who have attempted to identify large collections of isolates from complex natural sources such as soil. Strain differences within species, various species complexes, inadequate description of forms in the literature, and failure of some isolates to sporulate may be mentioned as some of the obstacles encountered. It is certain that a few of the species dealt with have not previously been described. However, even where species determinations could not be made, a majority of the cultures could be successfully sorted into what appeared to be specific entities, on the basis of distinctive cultural and morphological features. A few forms initially undetermined were successfully placed at a later date when more intensively studied. Unidentified and unidentifiable isolates recognizable as entities were assigned numbers and dealt with on that basis. Finally, for all samples of a given date for a

given forest stand, the frequency and density percentages of the principal species represented were calculated and tabulated on marginal punch cards. The analytical determinations were computed as follows:

$$\text{Frequency percent} = \frac{\text{Number of samples of occurrence}}{\text{Total number of samples}} \times 100$$

$$\text{Density} = \frac{\text{Total number of cultures of a species}}{\text{Total number of cultures of all species}} \times 100$$

Cultural Methods Employed in Identification

Identification of the fungal isolates was made or attempted from a study of these organisms growing on various media in Petri dish culture. A standardized procedure, adopted from Thom and Raper (**8**) was employed in connection with the penicillia and aspergilli. This involved the use of Czapek's solution agar, corn steep agar, and malt extract agar as described by Thom and Raper (**8**) and Raper and Thom (**6**).[3] Potato-dextrose agar, made according to the formula of Riker and Riker (**7**), and Cutter's synthetic medium, "M.D.A. agar" (**2, 5**), were employed in the identification of the Mucorales. In the study of other fungi potato-dextrose agar and cornmeal agar[4] were used almost exclusively.

A stock culture reference collection, including all determined species and undetermined entities believed to represent species was established and added to as the study progressed. Tube slants of potato-dextrose agar were employed for all active stock cultures; these stocks were generally kept under refrigeration and transfers were made at appropriate intervals. In addition, supplementary stock cultures of certain of the organisms were set up as soil preparations, utilizing a modification of the technique described by Greene and Fred (**4**). These soil stocks were stored at room temperature.

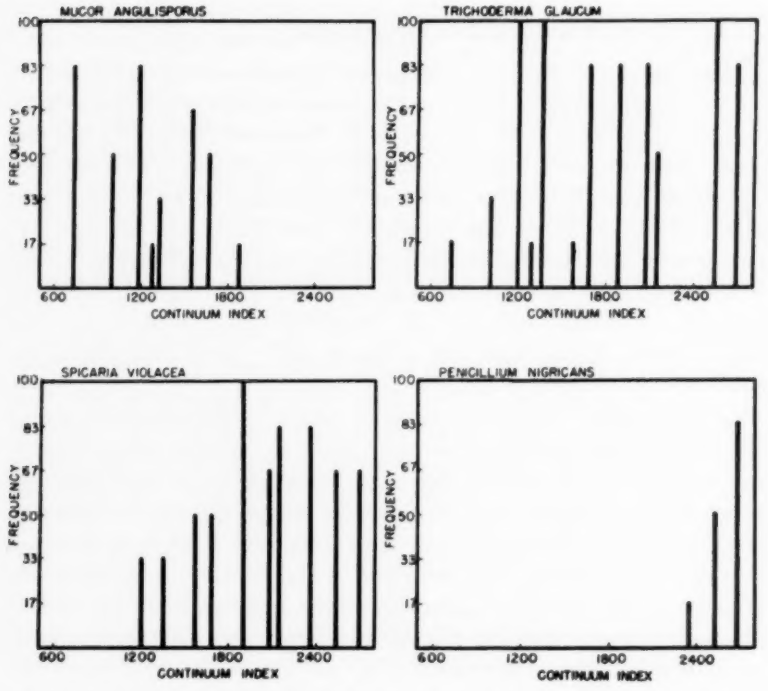
RESULTS

Because many of the fungi isolated in the course of this investigation were of low incidence and appeared only sporadically, also because the time available was inadequate for intensive identification work on the

[3] Dr. K. B. Raper kindly determined certain isolates of *Penicillium* which were submitted to him and checked the writers' determinations of some others.

[4] Filtered infusion of corn meal with 20 gms agar per liter. The infusion was prepared by steeping 125 gms yellow corn meal in 3 liters of lake water on a water bath at 58° C for 1 hour.

entire array of cultures obtained, it was decided early in the program to limit detailed attention to those species most commonly encountered. The arbitrary criterion set up required that a fungus, to receive consideration in the final detailed analyses, must ordinarily have shown a frequency of 50 percent or greater in some stand sampled or have been found in four or more sampling areas. Most forms not fulfilling these



FIGS. 1–4. Frequency percentage of selected species arranged according to continuum index of stands. 1 (upper left). A pioneer species. 2 (upper right). A species of broad tolerance. 3 (lower left). A c'imax species of moderate range. 4 (lower right). A climax species of the "indicator" type.

requirements were ultimately eliminated from the stock culture collection. Approximately seventy-five species met the requirements set up.

In the consolidation of the data which have been amassed from the various aspects of the study, certain well-defined patterns and relationships have been observed.

Species Distribution

The distributional patterns of the soil microfungal species were found to be remarkably similar to some shown by Curtis and McIntosh (1) to exist among the higher plants. This resemblance was particularly noticeable between the microfungi and the herbaceous plants; i.e., some species of soil microfungi were restricted to a particular segment of the forest continuum, whereas others occurred throughout the entire range. The range of the continuum over which a particular species was distributed and the point at which optimum development occurred became quite well defined when the frequencies of the organism were arranged along the gradient of the continuum (FIGS. 1–4).

The species were characterized according to the position in the continuum where they reached their crest of frequency. *Spicaria violacea* (FIG. 3), which reached its maximum in the more mesic or climax portion of the continuum, was characterized as a "climax" species even though its range extended well into the more pioneer portion. Similarly, *Mucor angulisporus* (FIG. 1) was termed a "pioneer" species on the basis of the location of its frequency maximum in the continuum. *Penicillium implicatum* was able to tolerate a rather broad intermediate range of the continuum, but reached its peak of frequency in the more climax stands, and thus has been considered a climax species.

The range of environmental tolerance of some species has been observed to be relatively narrow and such species serve as excellent indicators of a particular soil environment. As examples, *Penicillium janthinellum* and *Mucor angulisporus* (FIG. 1) were representative of the soils of pioneer stands, while *Penicillium nigricans* (FIG. 4) and *Spicaria* sp. (14–13) were indicative of soils found in climax stands. Such soil microfungal species have been found to be relatively sensitive indicators and may vie in importance with higher plants as indicators of soil environment.

In contrast to the foregoing type of distributional behavior a few species were found throughout the entire range of the forest continuum, but with highest frequencies in the central portion of the continuum (*Trichoderma glaucum*—FIG. 2). *Penicillium raistrickii* and a species of *Tilachlidium* (No. 15–15) were likewise widespread in the stands sampled but never occurred with high frequencies. The distribution of other fungi through the thirteen hardwood forest stands studied is given in TABLE I. The data presented were compiled in the survey made during the summer months.

In the foregoing examples the commonness of occurrence of the various

TABLE I

FREQUENCY* OF FUNGI IN SOILS† OF THIRTEEN FOREST STANDS

Species‡	C.I.§ 747	C.I. 1004	C.I. 1192	C.I. 1288	C.I. 1336	C.I. 1561	C.I. 1682	C.I. 1881	C.I. 2062	C.I. 2127	C.I. 2352	C.I. 2517	C.I. 2680
Haplosporangium sp. (7–5)		83											
Spicaria sp. (10–2)		17	33										
Chaetomium sp. (40–4)	50			67									
Haplosporangium sp. (25–7)	17					50							
Penicillium janthinellum	100	83	83	50	100	50							
Penicillium waksmani	50	17					83						
Mortierella sp. (8–1)	33	67	67		67		83						
Oospora sulphurea	17	17	17		67		33						
Mucor angulisporus	83	50	83	17	33	67	50	17					
Mucor ramannianus	100	33	17	33	50	50		17					
Monilia sp. (40–3)	50		17		33		17	17					
Mesobotrys sp. (9–2)						17		17					
Cylindrocarpon sp. (9–3)			33					33					
Sp. indet. (24–9)				67		50		17					
Cephalosporium sp. (10–3)	50	67	50		33	100			17				
Absidia cylindrospora	17	67	33		50		50			17	67		
Penicillium simplicissimum	83	17	83	50	67	67	100	50		67	17	50	
Phoma sp. (18–5)		17						17				33	
Tilachlidium sp. (15–15)	33	17	17		17	17	17	33	33	33	33	17	
Cladosporium herbarum	17			33	50				50	50	17	50	17
Sp. indet. (13–5)	33	33		83	50	50		33	17	67			33
Myrothecium verrucaria	17	50			50	50		33	83		17		50

* In per cent. A blank in the table signifies that the given species was not found in any of the six samples from the forest in question.

† Data based on summer sampling of A_1 layer as described in text.

‡ Except for *Mucor angulisporus* (Naumov) Naumov and *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr., the authorities for all binomials in this list are as given by Gilman (**3**) or by Raper & Thom (**6**).

§ C.I. Continuum Index number.

TABLE I—*Continued*

Species‡	C.I.§ 747	C.I. 1004	C.I. 1192	C.I. 1288	C.I. 1336	C.I. 1561	C.I. 1682	C.I. 1881	C.I. 2062	C.I. 2127	C.I. 2352	C.I. 2517	C.I. 2680
Penicillium lanosum	50			67					17	100			33
Penicillium raistrickii	17		33	33	17			17			33	33	17
Trichoderma glaucum	17	33	100	17	100	17	83	83	83	50	100		83
Trichoderma koningi	67	83	17	17	33	33	33		33	17	50		33
Trichoderma lignorum	33	67	67	83	17	33			17	100	17	17	50
Sp. indet. (16–2)	67								33	17			17
Mucor hiemalis		33				17			17			17	17
Penicillium implicatum			17	100	50	50	50	17	100	17	100	83	33
Spicaria violacea			33		33	50	50	100	67	83	83	67	67
Verticillium sp. (17–4)			17	50	33	17		100	17		33	50	83
Penicillium funiculosum				50				50			17		17
Penicillium restrictum				83		17		67	17	100		83	33
Cylindrocarpon sp. (47–5)				17	17							83	
Sporotrichum sp. (14–15)				17		17		50		83		50	50
Zygorhynchus vuilleminii				17	17						33		
Penicillium granulatum					33		17		83		50	17	17
Penicillium herequei						17			17		83	50	17
Penicillium sp. (25–14)						67							
Absidia glauca						17		33	50		33	17	50
Pencillium steckii						67							
Penicillium variabile							50		83		67		17
Penicillium spinulosum							83				33		50
Penicillium stoloniferum							17		67		33	33	50
Spicaria sp. (14–6)								17					17
Spicaria sp. (14–13)									83		83	33	83
Volutella sp. (13–9)									50			17	50
Penicillium nigricans											17	50	83
Penicillium sp. (17–3)											67	50	
Penicillium admetzi												67	
Cylindrophora sp. (57–6)												50	
Penicillium thomii												33	33

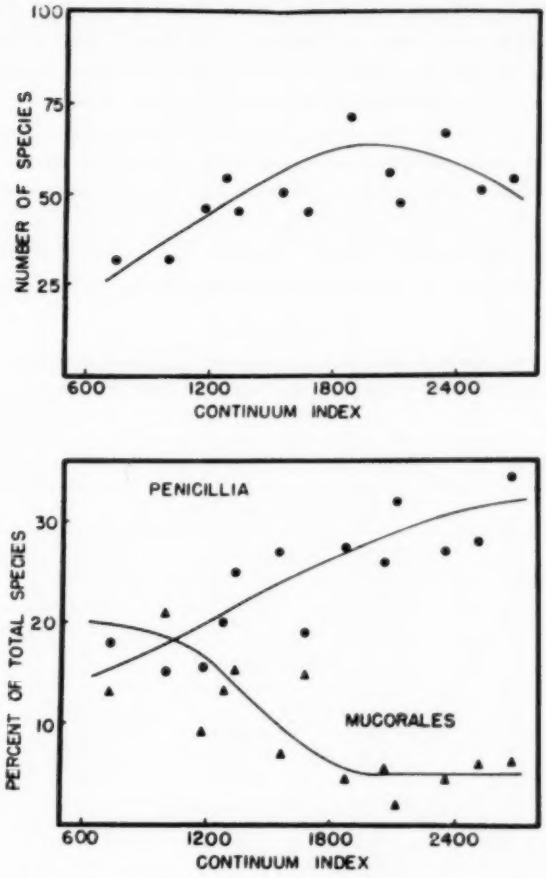
microfungal species was assessed on the basis of frequency. Such a measurement well expresses how widely a species is distributed, but does not present a picture of the relationship of the number of individuals of one species to another. By computing the relative density for all the species in each stand, an approximation of this relationship was obtained. It can be argued that relative density is not a satisfactory character for measuring soil microfungi, since individual colonies appearing on the isolation plates may result from either fungal spores or mycelial fragments; thus, instead of measuring the ratio of active individuals of one species to another, one might be measuring their sporulation capacities. This is undoubtedly a valid objection. However, it has been observed that of two species possessing similar sporulation capacities in culture, the relative densities of one may be consistently high whereas those of the other may be consistently low. Furthermore, species which are not particularly heavy sporulators may quite constantly have high relative densities. Therefore, it seems justifiable that some significance be attached to relative density as an analytical character.

When the distributional patterns as determined by relative density and frequency are compared, it is found that the peak of development for some species is more sharply defined by density than by frequency, whereas in other instances the two curves coincide quite closely and the density curve substantiates the developmental peaks shown by frequency.

Number of Species and Special Groups of Microfungi

It was commonly observed that soils from pioneer forests generally yielded a relatively low number of species, while climax forest soils contained a considerably higher number. However, when the number of species per stand was plotted along the gradient of the forest continuum, the greatest number of species occurred not in the most climax stands, but in the range between 1800 and 2400 of the forest continuum (FIG. 5). The explanation of this phenomenon may lie in the overlapping of ranges of some pioneer and climax species within this segment. A similar phenomenon has been observed to occur with the herbaceous species of higher plants.

Not only has the soil environment along the forest continuum determined the number and kinds of species present, but its influence has been reflected in the distribution of entire groups of microorganisms such as the penicillia and Mucorales. The percentage of penicillia in the soil was found to increase steadily from the pioneer to the climax stands, whereas the percentage of Mucorales fell off rapidly in this direction (FIG. 6). This would indicate opposite adaptations to the same soil



FIGS. 5 and 6. 5 (above). Total number of species isolated per stand. 6 (below). Relative proportion of penicillia and Mucorales along continuum index.

environment. The aspergilli as a group were poorly represented in all forest soils studies. Yeasts, likewise, were not very abundant.

Seasonal Influences on the Soil Microorganisms

In the forests of southern Wisconsin extreme seasonal changes may occur in soil temperature, soil moisture and even soil organic matter content; however, the magnitude of the fluctuations may vary in the different forest types of the continuum.

Two dissimilar forest types were chosen for a study of some of the seasonal influences on the soil microfungal flora—one, a pioneer oak forest with a continuum index value of 1004, the other a more climax maple-basswood stand with an index of 2517.

Shown in TABLE II, the seasonal variation in numbers of soil microorganisms in most instances correlated well with the moisture percent of the soil, although other factors undoubtedly contributed to the size of their populations. The increase in winter and spring populations was perhaps also conditioned by the increase in available organic matter supplied to the soil by the leaf fall during the previous autumn. The

TABLE II

SEASONAL EFFECT ON NUMBER OF MICROORGANISMS*

	Number of microorganisms per gram dry soil			
	Spring	Summer	Autumn	Winter**
Maple-Basswood Forest				
Bacteria	58,400,000	40,500,000	23,500,000	55,100,000
Actinomycetes	4,800,000	2,800,000	2,200,000	2,700,000
Fungi	452,000	282,000	258,000	437,000
Soil Moisture %	41.4	29.1	25.9	36.4
Oak Forest				
Bacteria	27,400,000	13,200,000	13,400,000	40,100,000
Actinomycetes	3,800,000	2,300,000	1,600,000	1,200,000
Fungi	731,000	296,000	492,000	651,000
Soil Moisture %	39.7	29.9	31.4	31.3

* Studies made during Autumn 1950, Winter 1950–51, Spring 1951, and Summer 1951.

** Soil unfrozen under a heavy snow cover.

reduction in numbers during the summer was probably, then, a result of a decrease in both available organic matter and soil moisture. Apparently temperature played only a minor rôle in determining the size of the bacterial and fungal populations, since the winter populations were always greater than those in summer; the actinomycetes, however, showed a reversal in this trend. It is of interest to note that the numbers of bacteria and actinomycetes were consistently higher during each season in the soil of the maple-basswood stand than in the soil of the oak stand during the corresponding season. The fungi, on the other hand, apparently were more favored by the soil environment of the oak stand, because larger populations were found here during all seasons

than in the maple-basswood stand. The greater acidity of the soil in the oak stand undoubtedly favored the fungi. At the same time it provided a less favorable environment for the bacteria and actinomycetes and probably played a part in limiting their numbers.

TABLE III

FREQUENCY* OF VARIOUS FUNGI IN THE SOIL** OF AN OAK FOREST (C.I. 1004) AND OF A MAPLE–BASSWOOD FOREST (C.I. 2517) AT DIFFERENT SEASONS

	Oak				Maple-Basswood			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Haplosporangium sp. (7–5)	100	83	83	50	0	0	0	0
Mortierella sp. (8–1)	83	67	83	100	0	0	0	0
Mucor angulisporus	83	50	67	67	0	0	0	0
Mucor ramannianus	67	33	83	33	0	0	0	0
Oospora sulphurea	67	17	67	83	0	0	0	0
Penicillium janthinellum	100	83	100	83	0	0	0	0
Spicaria sp. (10–2)	33	17	33	100	0	0	0	0
Cephalosporium sp. (10–3)	33	67	67	0	0	0	0	0
Cylindrocarpon sp. (9–3)	16	0	16	83	0	0	0	0
Penicillium waksmani	67	17	33	0	0	0	0	0
Mesobotrys sp. (9–2)	17	0	0	17	0	0	0	0
Absidia cylindrospora	100	67	100	83	0	0	33	0
Penicillium simplicissimum	83	17	83	33	0	50	17	0
Myrothecium verrucaria	0	50	33	50	50	0	50	17
Trichoderma lignorum	17	67	83	100	33	17	67	33
Phoma sp. (18–5)	17	17	0	0	17	33	0	0
Trichoderma glaucum	50	33	0	0	67	50	17	83
Tilachlidium sp. (15–15)	0	17	0	17	50	17	0	17
Penicillium raistrickii	17	0	17	0	0	33	0	17
Penicillium purpurogenum	0	17	0	17	17	17	50	33
Verticillium sp. (13–3)	0	0	0	50	33	0	33	0
Mucor hiemalis	0	33	0	0	50	17	17	33
Cylindrocarpon sp. (47–5)	0	0	0	0	0	83	50	0
Penicillium adametzi	0	0	0	0	0	67	0	50
Sporotrichum sp. (14–15)	0	0	0	0	50	50	0	0
Penicillium martensii	0	0	0	0	33	0	33	17
Penicillium roseo-purpureum	0	0	0	0	17	33	0	17
Sp. indet. (15–14)	0	0	0	0	33	0	50	67
Penicillium nigricans	0	0	0	0	17	50	100	0
Absidia glauca	0	0	0	0	17	17	17	100
Penicillium sp. (17–3)	0	0	0	0	17	50	83	33
Penicillium herequei	0	0	0	0	50	50	100	33
Penicillium implicatum	0	0	0	0	67	83	100	83
Penicillium stoloniferum	0	0	0	0	17	33	33	33
Penicillium thomii	0	0	0	0	17	33	33	33
Spicaria sp. (14–13)	0	0	0	0	83	33	100	83
Spicaria violacea	0	0	0	0	83	67	100	100
Verticillium sp. (17–4)	0	0	0	0	33	50	83	83
Volutella sp. (13–9)	0	0	0	0	83	17	50	50

* In percent.
** A_1 layer, sampled as described in text.

Frequency and relative density were employed in studying the seasonal populations of the more common soil microfungal species in the aforementioned oak and maple-basswood stands. The data showed marked seasonal frequency and density maxima for some species while the total number of species per stand remained quite constant and the same species were encountered at essentially all seasons (TABLE III). The percentage of penicillia and Mucorales in each stand was remarkably stable throughout the year.

Soil Profile Studies

In addition to the investigation of the horizontal distribution of the soil microflora, an exploratory study was carried out on the aerobic populations in the soil profiles of the oak and maple-basswood stands mentioned above.

The soil of the oak stand was a sandy loam of loessial origin and possessed a well-defined profile. The A_0 horizon consisted of a layer of partly decomposed leaf litter about ¾ of an inch deep; the A_1, about 2 inches of dark, incorporated organic matter; the A_2, a 3-inch layer of lighter-colored, chalky leached soil; the B horizon, about 7 inches of enriched sandy mineral soil; and the C horizon, a mixture of fine sand and fragments of parent rock. The soil of the maple-basswood stand was a silt loam with a less well-defined profile. The A_0 horizon consisted of partly decomposed organic matter only about ¼ inch deep; the A_1 horizon, a layer of dark organic soil roughly 6 inches deep; the lighter A_2 layer was poorly defined, but approximately 4 inches thick; the mineral enriched B horizon was about 11 inches in depth and intergraded with the red-brown clay of the C horizon which extended for an undetermined depth below.

In general agreement with what has been reported by others who have examined the vertical distribution of microorganisms in the soil, it was found that fungal, bacterial, and actinomycete populations declined rather rapidly in samples taken at progressively lower soil depths in both the pioneer and climax forest. At the lowest level sampled—23 inches below the surface in the maple-basswood soil—bacteria numbered only 725 thousand, actinomycetes forty-eight thousand, and fungi eight hundred per gram of dry soil. The bacterial and actinomycete populations in the forest soil types appeared to be correlated with the organic matter gradient. Thus in the maple-basswood soil, with its thick A_1 horizon, the bacterial and actinomycete populations decreased more slowly through the upper six inches than was the case in the pioneer forest soil. On

the other hand, the rate of decline of the fungal populations did not seem to be strongly influenced by the organic matter gradient.

No pronounced differences in the kinds of microfungi isolated from the various soil depths were noted. In general, those species which were most commonly encountered in the surface layer of the soil were also found throughout most of the profile. However, at the lowest depths sampled some of these species were absent and were replaced to some extent by a few undetermined forms which were extremely slow-growing on the culture media employed.

DISCUSSION AND CONCLUSIONS

The first point to be emphasized here is that the authors fully recognize that they have dealt with only a limited segment of the fungal flora of the soils studied—namely, that portion which was revealed by the isolation methods employed. It is hoped that at a later date a more thorough study can be made. However, there can be little doubt that the species which were isolated are some of the most prominent members of the soil fungal populations in the forests in question.

As the writers view the situation, the significance of the present study lies not so much in the catalogue of species found in the southern Wisconsin hardwood forests as in the demonstration of a new approach to the study of the ecology of soil organisms. In the investigation conducted, the sampling sites were defined with great precision with regard to the higher plant cover; samples were taken and worked up in such a fashion that the data could be analyzed quantitatively. Too often in the past the area under study has been vaguely defined. Such terms as a "meadow" soil, a "pasture" soil, or simply a "forest" soil actually hold little meaning since they do not describe a definite type of habitat. And too often the studies undertaken have yielded only a bare list of species. It is doubtful whether the distributional patterns of the microfungi which emerged in the present study would have been revealed had the usual method of species compilation been used.

At this point attention must be called to a very interesting study recently carried out in England by Warcup (**9**) on certain undisturbed grassland soils. The paper in which this investigation was reported came to the writers' laboratory just as the present study was being brought to completion. Although a very different isolation technique was followed in the British work and although entirely different types of terrain were involved in the study, there was, in the approach to the problem, considerable resemblance to the pattern of operation followed in the present investigation. In the British work, as in the case at hand,

the areas sampled were precisely defined ecologically, seasonal influences on the microfungal flora were explored, the frequencies of the commonest species were plotted, and vertical as well as horizontal distribution of the microorganisms was considered. The results of both studies suggest that qualitatively, at least, fungal populations in the soil of undisturbed areas probably do not fluctuate much through the year. They both indicate, however, that the character of the microfungal population does vary considerably from one ecological area to another and that the distribution of the various species is influenced by higher plant cover, the amount and character of organic matter, and other factors. While individual actinomycete and bacterial species were not considered in either study, it would be surprising indeed if these are not also eventually found to follow similar distribution patterns.

The diversity of soil environment in the different plant communities in southern Wisconsin has become increasingly apparent. Just as the higher plant constituents of these communities presented a continuously shifting series of combinations of species in a definite sequence, the soil, being largely a product of the vegetation, likewise varied accordingly. No two soil habitats had identical physical and chemical characteristics, but instead they presented a continuously changing set of features dependingly largely upon the nature of the plant cover. In turn the microbial populations, which were directly controlled by the soil factors, reflected both qualitatively and quantitatively this changing environment.

An interesting parallel was found to exist between the microfungal populations and the vegetation of the higher plant communities with which these populations were associated. Well marked ranges of environmental tolerance were exhibited by many of the microfungal species studied, yet none of the species exhibited identical patterns. Also in the different soil habitats, different combinations of species occurred together. The more nearly alike the two habitats, the more species there were in common. Apparently no two habitats had exactly the same combination of species, but instead a progressively changing series of species combinations occurred throughout the forest continuum. Thus, it may be concluded that, analogous with the situation found by Curtis and McIntosh (1) for the higher vegetation in southern Wisconsin, no discrete soil microfungal communities exist in that region.

The seasonal changes which were observed to occur in the microbial populations of the forest soils studied were apparently largely conditioned by soil moisture and available organic matter content. Insofar as could be determined, there were no significant seasonal species changes among the most important members of the fungal population, but instead only fluctuations in the number of species of minor importance.

The studies of the vertical distribution of microfungal species in the soils showed that species which were most common in the surface layers were generally found throughout all except the lowest layers sampled. This suggests that many of the most common species possess a wide range of tolerance for reduced moisture, low organic matter content, and low oxygen relations generally characterizing the lower depths, and that these species do not disappear completely until one or more of these factors become limiting. The possibility also exists, however, that at least some of the colonies obtained from samples taken well below the surface may have grown from spores which were produced near the surface and had simply been carried downward with the percolating water.

The present type of investigation appears to hold great future promise in the field of microfungal ecology. Because the approach employed here yields a type of information not readily obtained by any of the methods of study commonly hitherto employed, it is believed that continued work along this line can eventually give a more comprehensive ecological picture of the soil micro-inhabitants and, especially, it promises to shed increased light on the complex interrelationships existing between the higher plant cover and the soil microfungal populations.

SUMMARY

A survey study was made of the microfungal flora of the soils in a series of upland hardwood forests in southern Wisconsin. These forests constituted a vegetational continuum which was composed of a continuously shifting series of combinations of tree species in a definite sequence that ranged from groupings of more pioneer species at one extremity of the series to more climax assemblages at the other.

The analytical measures, frequency and relative density, commonly used for analyzing higher vegetation, were applied to the measurement of the microfungal flora. The size of the accompanying bacterial and actinomycete populations was also determined.

When the average frequencies of the microfungal species were plotted along the gradient of the forest continuum, the range of the continuum over which a species was distributed and the point of its optimum development were revealed. Characterized according to the position in the continuum in which they reached their crest of frequency, *Spicaria violacea* and *Penicillium nigricans* were typical "climax" species; *Penicillium janthinellum*, *Mucor ramannianus*, and *Oospora sulphurea* were "pioneer" species, and *Penicillium granulatum* was an "intermediate" type. The restricted distributional ranges of *P. janthinellum*, *P. nigri-*

cans, Mucor angulisporus, and *Spicaria* sp. (14–13) have rendered these species valuable as "indicators" of certain soil environments.

Relative density, when compared with frequency, either more sharply defined the optimum range of development of a species or confirmed the range shown by frequency.

The number of species of microfungi was found to increase from the pioneer toward the climax end of the continuum; as a result of the overlapping of ranges of both pioneer and climax forms, the greatest number of species occurred in stands somewhat preceding the most climax in the continuum.

There was a steady increase in the percentage of penicillia in the soil from the pioneer to the climax forests, while the reverse of this trend was true for the Mucorales. The aspergilli were poorly represented in all forest soils studied.

The size of the soil microfloral populations correlated well with the moisture and organic matter content of the soil. The bacterial and fungal constituents were not markedly affected by soil temperature, since they were higher in winter than in summer; the actinomycetes, however, showed an opposite trend.

During each season the numbers of bacteria and actinomycetes were consistently higher in the soil of a maple-basswood stand than in the soil of an oak stand; however, during these same periods the microfungal population was greater in the oak woods soil.

Although there was little seasonal change in the species of microfungi present in the soils, there were seasonal density and frequency maxima shown for some species.

In an exploratory study of the vertical distribution of microorganisms in the soils of two forest stands at opposite ends of the continuum, the species of microfungi most commonly encountered in the surface layers were also found throughout most of the profile; however, at the lowest depths sampled they were replaced in part by a few undetermined species characterized by slow growth.

It is concluded that, analogous with the situation existing in the higher plant cover in southern Wisconsin, the forest soil microfungi do not form discrete communities in which particular forms are consistently associated; instead, a series of progressively changing species combinations is found along the gradient of the forest continuum.

DEPARTMENT OF BOTANY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

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ISOLATION OF MYXOTRICHUM AND GYMNOASCUS FROM THE LUNGS OF ANIMALS [1]

CHESTER W. EMMONS

(WITH 4 FIGURES)

In attempting to delineate the endemic areas of coccidioidomycosis in western Texas, cultures were made in 1949 from the lungs, spleen and liver of 764 rodents and other animals,[2] some of which are known to be natural hosts of *Coccidioides immitis* (2). Although *Coccidioides* was not isolated from animals in the particular area under study, another pathogen, *Haplosporangium parvum*, was isolated from the lungs of 7 animals.

Among the saprophytic fungi isolated also from the lungs of these animals were 9 strains of *Myxotrichum* sp. and 3 strains of *Gymnoascus reessii* which attracted attention because of their microscopic resemblance to *Coccidioides*, the fungus being sought. Seven of the strains of *Myxotrichum* were isolated from the wood rat (*Neotoma micropus*), one from a ground squirrel (*Citellus mexicanus*) and one from a cotton-tail rabbit (*Sylvilagus auduboni*). The strains of *Gymnoascus* were isolated from the wood rat and the prairie dog (*Cynomys ludovicianus*). Although the isolations were from the lungs, no pulmonary lesions were detected, and the fungi do not produce disease in the experimentally infected mouse. Therefore it is assumed that the fungi isolated were represented in the animals by recently inhaled spores or were airborne contaminants of the cultures, which were made under dusty field conditions. These lower Ascomycetes are generally known as dung-inhabiting fungi and it is to be expected that their spores will be in soil in the vicinity of animal burrows.

[1] From the U. S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Microbiological Institute, Laboratory of Infectious Diseases, Bethesda, Md.

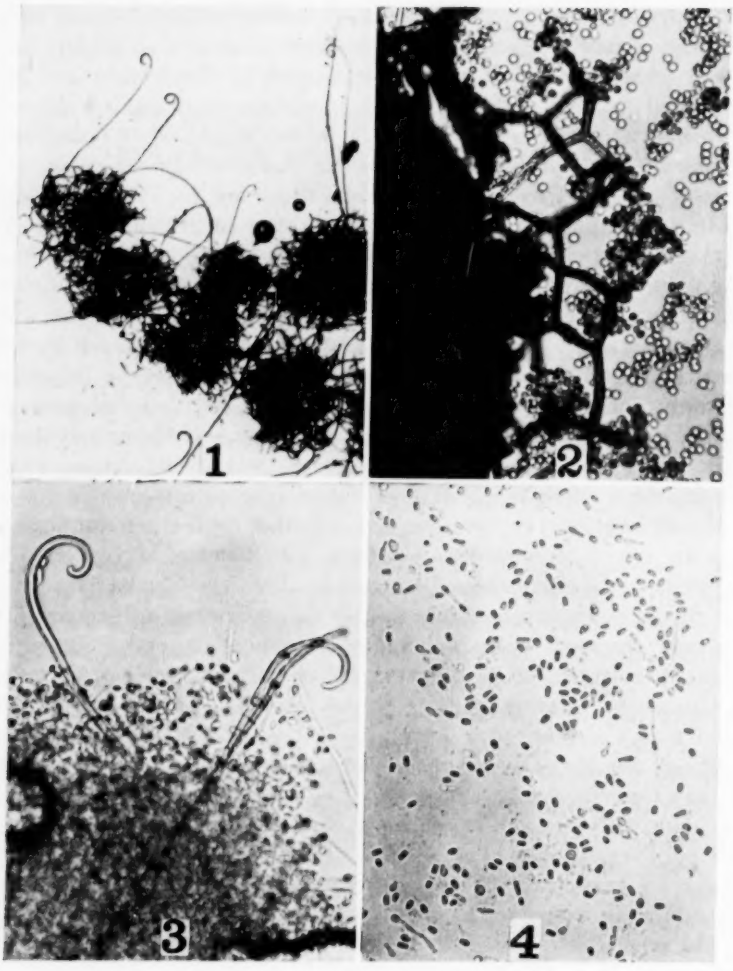
[2] Animals in this series were collected with the cooperation and assistance of Dr. Carl Mohr, Director, Mr. Virgil I. Miles and other members of the Plague Typhus Control Group, U.S.P.H.S., from Andrews, Cochran, Dawson, Ector, Gaines, Hockley, Lubbock, Midland and Yocum Counties, Texas.

Myxotrichum and *Gymnoascus* are of medical interest because they bear large numbers of arthrospores similar to those of *Coccidioides* and thereby present a problem in the identification of *Coccidioides* and the differential diagnosis of disease. It is not uncommon, in making cultures of sputum as a laboratory procedure in the diagnosis of pulmonary coccidioidomycosis, to isolate fungi which bear arthrospores closely resembling those of *Coccidioides*. Some of these are Basidiomycetes and can be so identified by the presence of "clamp connections." Several years ago I repeatedly isolated from sputum of one patient *Coprinus micaceous* which readily produced mushrooms in the culture flask. Other similar fungi present no specific characteristics by which I have been able to identify them, although some have the rather coarse, rapid growth characteristic of many Basidiomycetes, and others may be imperfect stages of members of the Gymnoascaceae. It is assumed that these, as well as many other saprophytic fungi appearing in cultures inoculated with sputum, come from spores which were recently inhaled or which fell into the specimen at the time of collection or subsequent thereto.

Myxotrichum and *Gymnoascus* are of further medical interest because they are able to survive mouse passage, i.e., they can be recovered in culture from the mouse tissues some weeks after experimental inoculation into the peritoneal cavity, although they do not produce progressive disease and observed lesions are limited to minute omental abscesses in which the inoculum has been sequestered and remains viable. A number of saprophytic fungi are capable of such survival. In this respect they differ from *Coccidioides*, which produces a rapidly fatal disease in mice. Although familiarity with *Coccidioides* usually permits the mycologist to identify *Coccidioides* with certainty, it is sometimes necessary to inoculate mice with the saprophytic fungi isolated from sputum in order to rule out the possibility of an atypical strain of *Coccidioides*. As will be shown, the examination of *Myxotrichum* upon suitable media makes this procedure unecessary for its identification.

The strains of *Gymnoascus* isolated do not present any unusual features and will not be discussed in detail.

The strains of *Myxotrichum* isolated resemble *M. uncinatum* (Eidam) Schroeter 1893 (**7**) but differ from it in color and spore dimensions. They were compared with the type species, *M. chartarum*, through the courtesy of John A. Stevenson, Curator, Mycological Collections, Bureau of Plant Industry. They differ from that species by more delicate structure and appendages which are longer and are not branched. They differ from other species for which adequate descriptions were found by dimensions of spores or shape of appendages (**6**).



FIGS. 1–4. *Myxotrichum* sp. 1. Ascocarps and appendages, × 70. 2. Peridium and ascospores, × 330. 3. Appendages and arthrospores in absence of ascocarps, × 300. 4. Arthrospores, × 300.

Corn meal agar induced abundant development of both arthrospores and ascocarps. The latter are at first pale yellow and become brown or reddish brown with age. Under the microscope they present the striking, loose, basket-like globose peridium composed of thick-walled, orange-yellow to reddish, freely branched, anastomosing hyphae characteristic

of the genus (FIGS. 1, 2). From the arched portions of these hyphae brown thick-walled appendages 200–500 μ long arise. These are approximately the same diameter and color as those making up the peridium but are smooth and unbranched, with usually a single septum near the base, and are straight except for the uncinate tip which characterizes this species. The tip may be simply hooked or it may make one complete turn, but it does not coil to the extent found in *M. chartarum*.

Production of these appendages persists in strains which, under cultivation in the laboratory, have ceased to produce ascocarps. In such strains they may arise singly and at random among the vegetative hyphae in the absence of any discernible ascocarps, but often with an associated increase in the production of arthrospores (FIG. 3). The persistence of these characteristic structures permits identification of otherwise atypical and puzzling strains.

The asci are evanescent. The ascospores are subglobose, 2.5–3 μ in diameter and minutely asperulate. Ascospores of one of the strains studied are spiny. The arthrospores are remarkably like those of *Coccidioides*. They are barrel-shaped and often alternate in the chain with empty spaces (FIG. 4), just as in *Coccidioides*. On glucose-neopeptone agar they are more elongated and cylindrical.

On glucose-neopeptone agar vegetative growth is much more profuse than on corn meal agar and the color of a fertile strain varies from golden-yellow to orange. Ascocarps are produced very rarely and there is diminished production of arthrospores. Non-fertile strains lose the normal pigment-producing ability.

DISCUSSION

In addition to the strains discussed above, which presumably came indirectly from soil, both *Myxotrichum* and *Gymnoascus* have been isolated directly from soil, by injection of soil suspensions into mice. These isolations and the isolation of several important pathogens of man from soil (**1**) focus attention upon the importance of the soil as a reservoir of fungi pathogenic or potentially pathogenic for man and upon the selective technic by which they can be isolated from this medium.

The systemic mycoses of man are not contagious and do not spread readily from host to host as in fungus diseases of plants or as in many infectious diseases of man. Application of the method of mouse inoculation has indicated that soil (perhaps specifically the highly nitrogenous component of soil arising from animal excreta and debris) is the most important source of infection for the systemic mycoses and perhaps for some of the dermatophytes (**1**, **3**, **4**, **5**).

SUMMARY

During a search for coccidioidomycosis in rodents in western Texas, 9 strains of *Myxotrichum* sp. and 3 of *Gymnoascus reessii* were isolated from the lungs of animals. They produced no apparent disease, but are able to survive mouse passage, and were selected from other saprophytes isolated because of microscopic resemblance to *Coccidioides*.

NATIONAL INSTITUTES OF HEALTH,
BETHESDA 14, MARYLAND

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ASCOGONIA AND SPERMATIA OF STEREOCAULON [1]

GEORGE THOMAS JOHNSON

(WITH 12 FIGURES)

Stereocaulon is a widely distributed genus of discomycetous lichens. Apothecial initials have been described in *S. paschale* (Wolff, **1905**) and in *S. coralloides* (Moreau and Moreau, **1932**). Otherwise, as Lamb (**1951**) has pointed out, little is known about the development of the apothecium in the genus.

The present paper is based on specimens from the *Sacculata* section of the genus (Satô, **1941**) collected by Mrs. M. S. Clemens on Mt. Kinabalu, in British North Borneo. These specimens do not possess mature apothecia and spores but have been assigned to *S. nesaeum* Nyl. on the basis of vegetative characters. The specimens permit a more complete description of the apothecial primordia, the ascogonia, and the thallus structure at the time these are present than is yet available for any species of *Stereocaulon*. Hence developmental stages from the Clemens collections were embedded in paraffin and celloidin, after which permanent slides were prepared for microscopic study in the usual manner. This material has been studied and the following observations made.

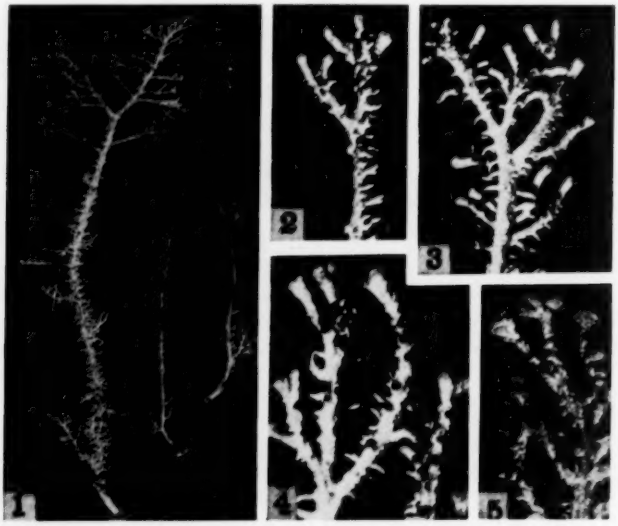
MORPHOLOGICAL OBSERVATIONS

Podetial morphology in *Stereocaulon* is relatively uniform and, except for apothecial details, these specimens of *S. nesaeum* are typical of the genus. Each mature podetium is erect, unbranched or with one or two long ascending branches below, but more abundantly branched above (FIG. 1). The podetium bears coralline phyllocladia, which are especially well developed and branch repeatedly if located near the base. The phyllocladia on the upper portion of the podetia are shorter, and still clearly coralline in nature, though sometimes appearing as papilliform granules. The podetia are decorticate below; in the upper branches three layers are normally present (shown in longitudinal section in FIGS. 8 and 10): (1) an outer cortex (*27–62 μ* thick), (*2*) a

[1] This study was initiated while the writer was a Fellow of the John Simon Guggenheim Memorial Foundation.

gonidial layer (in which the algae may occur in scattered groups or in a layer up to 30 μ thick), and (3) a central cylinder. The normal gonidium is *Protococcus;* typical scrobiculate cephalodia containing *Nostoc* also occur (cf. Johnson, **1938**).

Apothecia originate at the tips of podetial branches (FIGS. 8 and 12). Ascogonia were detected in many specimens where little enlargement of the branchlet had taken place (FIGS. 2 and 3). In young apothecial primordia when only a few ascogonia are present, each ascogonium is



FIGS. 1–5. *Stereocaulon nesaeum.* 1. Three podetia. 2, 3. Young apothecial initials. Well developed ascogonia provided with trichogynes occur within these terminal phyllocladia. Spermogonia can be observed in Fig. 3. 4. Intermediate stage in development of apothecial initial. Note abundant spermogonia. 5. Late stage in development of apothecial primordium. Approximate magnifications: Fig. 1, × ¾; Figs. 2–5, × 4.

fully developed, is about 11–15 μ in diameter, composed of a coil of cells, and provided with a trichogyne.

Shortly after the first ascogonia appear the branchlet increases noticeably in size and develops into a clavate or pyriform "head" (FIG. 4). Sections of such stages disclose a marked increase in the number of ascogonial coils (FIG. 12). The largest heads in the collection are from 3 to 4 mm broad (FIG. 5). They are peculiarly lobed or contoured and differ from the earliest stages both in texture and in color. The differ-

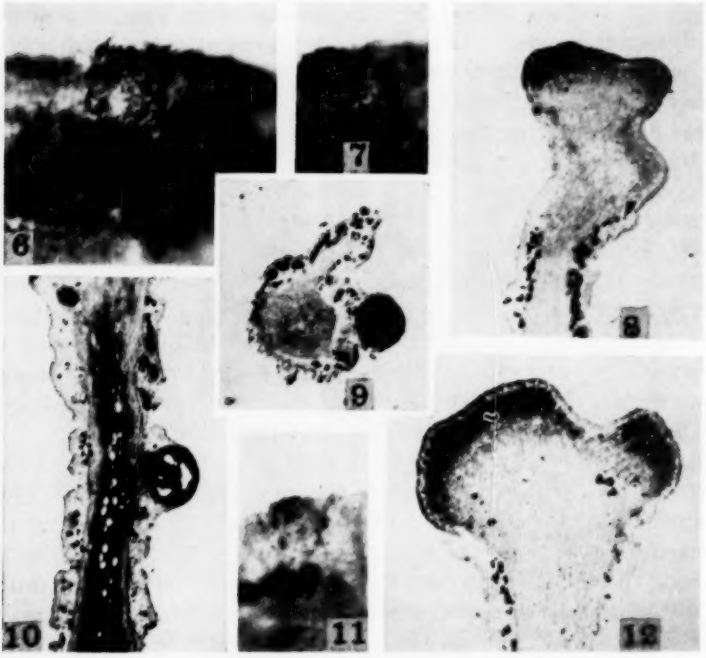
ence in color is primarily due to the absence of gonidia below the cortex; the difference in texture to the fact that the cortical hyphae above the ascogonia are smaller in diameter and less gelatinized than the usual cortical hyphae of the phyllocladia. At their maximum size these heads differ from the youngest primordia observed in the larger volume of the component parts, the lobed shape, the different color, and the larger number of ascogonia.

Cell wall outlines within the ascogonia are quite clear, but nuclear details cannot be seen. Ascogonial coils are composed of a fairly large number of cells and these are usually located from 30–110 μ below the surface of the head (FIGS. 6–8, 11–12). Several trichogynes may appear quite close together at the outer surface but only one trichogyne has been traced to each coil in all cases in which it has been possible to observe both structures in their entirety. Trichogynes not only connect the coil with the surface of the head, but they may extend as much as 22–48 μ into the air above (FIG. 6). Some trichogynes appear to stop near the surface, but the percentage which extends appreciably beyond is so high as to suggest that all trichogynes may do so at some stage in their development. The trichogyne which projected 22 μ beyond the surface was 31 μ from the surface to the coil. These and other measurements made indicate that a considerable portion of the total trichogyne may be exposed above the primordial head.

In older plants the ascogonia are quite numerous and located close together (FIG. 12). In *Pertusaria* Krabbe (**1882**) and Baur (**1901**) indicated that ascogonia may send out germinative processes and that new ascogonia may arise in nearby regions of the plant through such a method. FIGURE 11 illustrates a somewhat similar phenomenon within an apothecial head of the species studied. Several separate ascogonial coils connected by such deep-staining hyphae have been found within a single head.

Spermogonia are formed near the fertile tip (FIG. 4). They begin their development under the cortex of the uppermost phyllocladia just below the apothecial heads (FIGS. 3–5). The cortex is broken during their growth and they finally appear on the outer surface (FIGS. 9 and 10). Spermogonia and ascogonia are often produced in such close association that the spermogonia of one branchlet could come in direct contact with an apothecial initial on another branchlet as a result of slight movements brought about by the wind or other similar forces (FIG. 4). As many as eleven spermogonia have been observed within a length of 4 mm on a small phyllocladium. The spermogonia are dark brown in color, 240–300 μ across their widest parts, appear somewhat like peri-

thecia, and open by indefinite ostioles. Sections reveal typical spermogonial cavities with hymenia which are often irregularly lobed due to the fact that inward extensions of the spermogonial wall may divide the area into incomplete compartments (FIG. 10). Numerous arcuate-fusiform spermatia approximately 0.7–1 μ wide × 8–12 μ in length are produced.



FIGS. 6–12. *Stereocaulon nesacum*. 6, 7. Ascogonia with trichogynes. 8. Longitudinal section of apothecial head showing location of ascogonia. 9. Cross section of podetium showing spermogonium. 10. Longitudinal section of podetium showing spermogonium. 11. Ascogonium showing deep-staining hyphae which connect the ascogonium observed with a second one. 12. Longitudinal section of a relatively large apothecial head showing location of ascogonia. Approximate magnifications: Figs. 8–10 and 12, × 40; Figs. 6, 7 and 11, × 300.

Both spermogonia and ascogonia bearing trichogynes occur on the same plant at the same time. Free spermatia are produced which could be transferred easily to an apothecial head. Spermatia and trichogynes are thus closely associated though neither nuclear nor protoplasmic fusion has been observed. This means that only circumstantial evidence

is available, but the data suggest that spermatia have a sexual function in this species.

DISCUSSION

Wolff (**1905**) described apothecial initials in *S. paschale* but reported that no trichogynes could be observed. Judging from his figures, each apothecium developed from a single ascogonial coil. In *S. coralloides* the Moreaus (**1932**) found ascogonia surmounted with trichogynes. The trichogyne, however, was described as growing toward the surface of the head and degenerating and was not believed a functional organ. The apothecium was said to arise from one or more ascogonial groups, although the location of these structures was not clear and only a single detached group was figured.

The apothecial primordia of *S. paschale*, *S. coralloides* and *S. nesaeum* are similar in that ascogonial coils are formed, but the species studied by the writer differs from the descriptions of development in *S. paschale* and *S. coralloides* in that numerous ascogonia are formed near the surface of a rather extended primordium and that apothecia never arise from a single ascogonial coil. It has also been possible to observe a point not previously noted for the other species of this genus, i.e., that the trichogyne may project a considerable distance above the surface of the primordial head. Lamb (**1951**) recently proposed a classification of *Stereocaulon*, assigning *S. coralloides* and *S. paschale* to one subgenus and *S. nesaeum* to a different one. Possibly comparative morphological studies of apothecial development may eventually reinforce the other considerations upon which the taxonomic treatment of this genus has hitherto been based.

Spermogonia of *Stereocaulon* have been described and illustrated many times. Glück (**1899**) and Smith (**1921**) described them as concolorous with the podetia. Magnusson (**1926**) stated that two types may be present in the genus: one type immersed and with a pale wall, the other type sessile or stalked and with a dark brown wall. He had observed the latter type only in *S. denudatum*, *S. paschale* and *S. subcoralloides*. In the species studied by the writer the spermogonia are of the latter type and the walls are dark brown to brownish-black.

Wolff (**1905**) did not discuss trichogynes in *S. paschale* and stated that the apothecia were formed by purely vegetative processes. The Moreaus (**1932**) were unable to find spermogonia on *S. coralloides* when apothecial primordia were found. This, combined with the apparent degeneration of trichogynes they observed and the fact that ascogenous hyphae seemed to develop without a sexual fusion, led them to

regard spermatia as non-essential elements for spore formation in the genus. In addition to considering this evidence sufficient proof for the parthenogenetic development of ascogonia in *Stereocaulon*, the Moreaus considered the same type of evidence sufficient to support the same conclusion for other lichens that they studied. They then felt able to make the rather broad generalization that the development of ascogonia without spermatial stimulus is a character of sufficient phylogenetic importance to associate *Stereocaulon* with a series embracing the Collemaceae, Nephromaceae, Physciaceae, Stictaceae, Lecanoraceae, and some Pyrenocarpineae. It is evident that no validity should be attached to phylogenetic relationships indicated on the basis of a single character of this nature at the present time. Not only do the writer's observations, which indicate that spermatia function in a sexual process in one species of *Stereocaulon*, differ from those of Wolff and the Moreaus, who studied different species, but technical difficulties still prevent investigators from obtaining a clear picture of the nuclear phenomena involved in the production of sexual spores in most lichen species.

SUMMARY

In *Stereocaulon nesaeum* Nyl. ascogonia are first found in young apothecial primordia that do not differ macroscopically from other terminal phyllocladia. Numerous ascogonia develop as the primordia expand and deep-staining connecting hyphae uniting adjacent ascogonia have been observed. Each ascogonium develops a trichogyne which may project a considerable distance into the air. Spermogonia containing free spermatia are formed near apothecial initials bearing trichogynes. These observations suggest that spermatia have a sexual function in this species.

DEPARTMENT OF BOTANY AND BACTERIOLOGY,
UNIVERSITY OF ARKANSAS,
FAYETTEVILLE, ARKANSAS

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SPOT ANTHRACNOSE OF CHINESE HOLLY [1]

A. G. PLAKIDAS

(WITH 2 FIGURES)

In January, 1947, several Chinese holly trees (*Ilex cornuta* Lindl.) were found at Hammond, Louisiana, with numerous black spots on the upper surfaces of their leaves. Spotted leaves were collected and, by employing the technique found useful for isolating slow-growing fungi,[2] a *Sphaceloma* was readily isolated from the black lesions. Subsequent examinations of these trees revealed other symptoms, namely, bark lesions on the shoots (FIG. 1, c), scabby lesions on the berries (FIG. 1, d), and a large, elongated, dry, grayish black, scabby lesion occurring usually on one-half of the leaf blade and causing a severe distortion of the leaf (FIG. 1, b). The *Sphaceloma* was isolated many times from the lesions on the leaves, shoots, and berries.

ORIGIN OF THE DISEASE

As far as can be determined, this is a new and undescribed disease of this holly. No report of a spot anthracnose or any similar disease of this host has been found in the literature. The trees on which the disease was first observed were grown from seed that came from a tree on the Louisiana State University campus in Baton Rouge. Although the disease was later found on this tree, it seems very unlikely that the disease was transmitted by means of seed. Seed transmission of a spot anthracnose has as yet never been reported.

As there was a possibility that the disease had spread to the trees in Hammond from some infected host, a search was made in surrounding gardens and also in the neighboring woods where *Ilex opaca* and *I. vomitoria* abound, but the search was unsuccessful. Material (leaves and shoots) with suspicious lesions was collected from both cultivated and wild hollies and cultured, but in no case was a *Sphaceloma* isolated.

[1] In a preliminary report (Phytopath. **40**: 22, 1950) the disease was referred to as "scab," but it seems more appropriate to accept the suggestion of Jenkins (Plant Dis. Reptr. **31**: 71, 1947) and use the term "spot anthracnose."

[2] Plakidas, A. G. A convenient method for isolating slow-growing pathogenic fungi from plant tissue. Phytopath. **38**: 928–930. 1948.

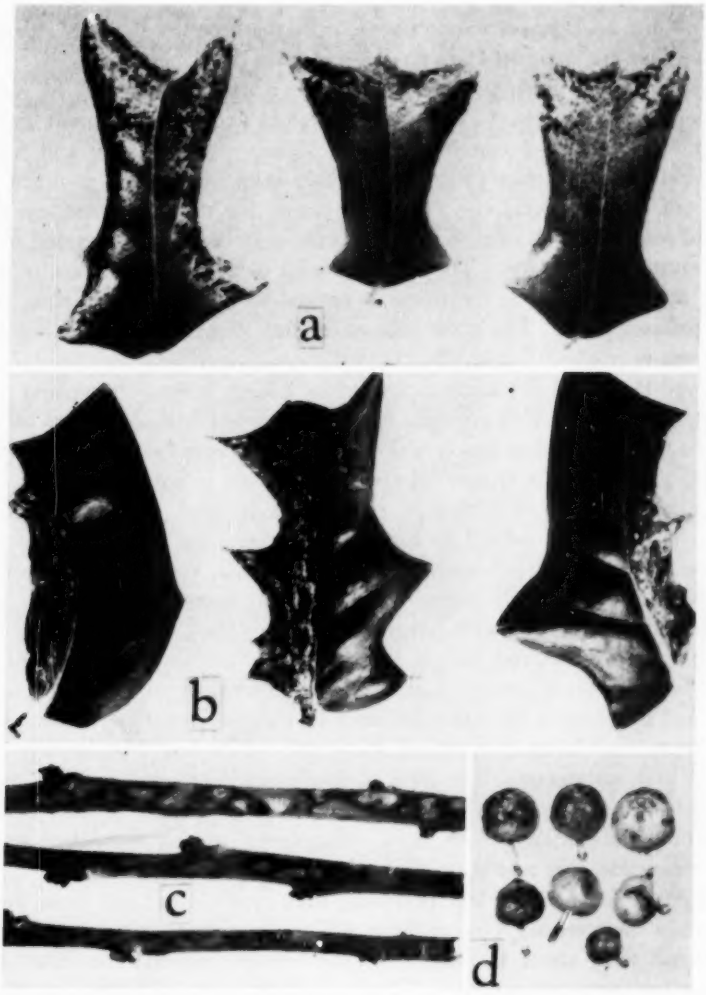

FIG. 1. A. Small black lesions, type A. B. Large, leaf-distorting lesion, type B. C. Bark lesions on shoots. Bottom shoot current season's growth with young lesions; other shoots previous season's growth with larger, coalescing lesions. D. Lesions on green berries.

DESCRIPTION OF THE DISEASE

Two distinct types of symptoms occur on the leaves. For convenience these types are designated as type A and type B.

Type A, the more prevalent of the two, is characterized by very numerous black spots. These spots, which at first are very small, gradually enlarge to 1–2 mm in diameter. They often coalesce and form irregular black patches (FIG. 1, a) which sometimes cover as much as 50% of the leaf surface. The spots occur, for the most part, on the apical one-half of the leaf, but occasionally may be evenly scattered over the entire leaf surface. They are confined to the upper surface of the leaf, and the injury to the tissue is limited to the upper epidermis and the palisade cells. The lower side of the leaf, directly below the lesions, appears normal.

Type B (FIG. 1, b) is characterized by a large lesion, often about 1½ inches in length, which typically is confined to one-half of the leaf blade. More than one lesion has occasionally been observed on the same leaf. Since growth of the tissues on the affected side is not normal, the leaf becomes markedly distorted in shape. As in type A, the lesions of type B also are confined to the upper surface, but the injury to the tissues extends deeper and sometimes becomes visible on the under surface. In the early stages, the affected areas are more or less chlorotic and marked with irregular brown patches. At this stage, the epidermis is intact and masses of subcuticular mycelium are present on the brown-colored areas. Later, the epidermis ruptures and sloughs off and the color of the lesion becomes dark gray. Still later, when most of the epidermis has disappeared, the surface of the lesion becomes rough, hard and carbonous. The alga, *Cephaleuros virescens*, is often associated with this type of lesion, but this seems incidental as this alga is commonly associated with spot anthracnose lesions on other hosts, including camellia and magnolia.

Type B infection is less prevalent than type A. It has been found on only three trees. One of these, a large, staminate tree, was severely affected with about one-fourth of the foliage spotted; the other two trees had only a few infected leaves. The severity of the disease on these trees did not change materially during a 5-year period.

Another important difference has been observed between types A and B, and that is the time of year in which symptoms appear on the new growth. Symptoms of type B appear in early summer and those of type A in late fall. During one year the new growth of affected trees was examined periodically from March until December. Type B lesions were found on the new leaves on July 11. As they were not present on

May 3, when the previous examination was made, they must have developed sometime between May 3 and July 11, probably during June. In contrast, black spot lesions (type A) were abundant and well developed on December 18, but not on October 17. They apparently developed sometime between these two dates, probably during November.

It is believed that the differences between the two types can be interpreted to be a host reaction. The trees were raised from seed, and they varied considerably as to size, habit of growth, and size and shape of the leaves. It is reasonable to think that they might also react differently to the pathogen.

Actually these trees fell within three distinct groups: those completely free of disease, those showing type A foliage symptoms, and those showing type B foliage symptoms.

The bark lesions on the shoots vary considerably with age. Young lesions (FIG. 1, c) on current season's growth are small, ½ to about 4 mm in diameter, flat or slightly raised, light brown in color at first, but later with grayish centers and light brown margins. Often a fine longitudinal crack occurs in the center of the lesion. Older lesions (on previous season's growth) are larger and they usually coalesce to cover large areas (½ to about 3 inches in length) of the shoot (FIG. 1, c). The margins of the older lesions are dark brown, and the centers gray. Numerous minute black specks (acervuli) occur on the gray portions of the lesions. Finally, the appearance of the older lesions is complicated by the growth of secondary fungi which invade the injured and dead tissue and also by that of the alga, *Cephaleuros virescens,* which often grows profusely on the affected bark.

The lesions on the green berries (FIG. 1, d) begin to appear in late June and become very prominent by the middle of July. They start as small brown spots, flat or slightly raised, later becoming corky, raised, and cracked. The cracks are sometimes fine, hardly visible to the naked eye, but may be large and prominent.

THE FUNGUS

In young lesions the mycelium occurs subcuticularly as thin, hyaline, interlaced but distinct hyphae. Later the mycelium invades the cells of the upper epidermis and becomes considerably gelatinized, making it difficult to differentiate individual hyphae. Disruption of the epidermal cells occurs, and the epidermis is partly dissolved and partly sloughed off, leaving the mycelium in the form of gelatinized stromatic layers attached to the outermost broken cells of the phellem.

Acervuli arise from the intraepidermal mycelium as raised, compact,

stromatic structures breaking through the cuticle (FIG. 2, a), and also from stromatic mycelium on the phellem after the epidermis has sloughed off. Neither well-differentiated conidiophores nor conidia were found in nature. Conidia often developed abundantly when, in the process of isolating the fungus, thin sections of tissue through acervuli were placed on hanging drops of carrot agar in Van Tieghem cells. These conidia developed from the tips of apical hyphae and not on differentiated

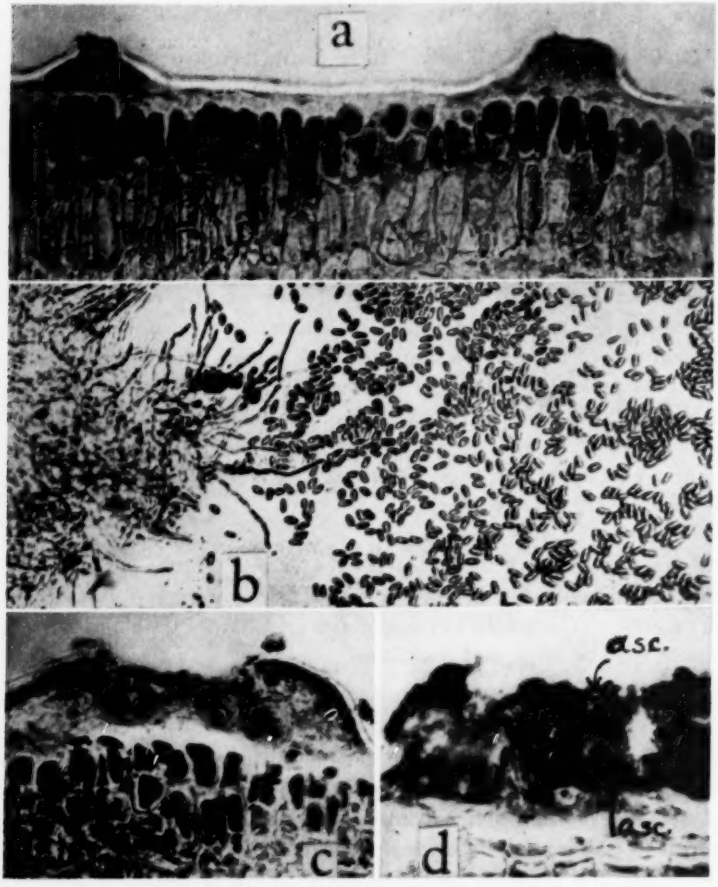

FIG. 2. A. Section through leaf lesions showing intraepidermal mycelium with 2 acervuli, × 300. B. Conidia produced in culture, × 390. C. Ascoma with several immature asci, × 300. D. Ascoma with mature ascospores (asc.), × 430.

conidiophores. Conidia also developed in culture when relatively young (7 to 10 days old) cultures on cornmeal agar were broken up into small fragments in a small amount of sterile water and the suspension was spread over the surface of solidified water agar in Petri dishes. Conidia were produced in great profusion (FIG. 2, b) within 7 to 24 hours, the greatest number within 12 hours. The conidia were formed terminally and laterally on hyphal cells which were indistinguishable from vegetative cells. It seems that the conidia are detached from the hyphae and shot off with considerable force because masses of them formed a scattered halo at a distance of several microns around the colony.

In a search for the perfect stage of the fungus, material comprising leaf and shoot lesions was collected at different seasons of the year (January, February, October, December) and examined both fresh and in paraffin sections. Ascomata, typical of *Elsinoë*, were frequently found, but these were immature for the most part (FIG. 2, c). A few mature asci were observed (FIG. 2, d). As only a few ascospores were found, it was not possible to determine with certainty the size or septation. The asci were irregularly imbedded in a stroma beneath the disrupted epidermis and were more or less pyriform in shape. The maximum number of ascospores seen in any ascus was 4.

The fungus grows slowly in culture. Colonies two weeks old on carrot dextrose agar ranged in size from 8 to 16 mm in diameter. The colonies were raised, cerebroconvolute, rubbery, and, for the most part, slimy. Some isolates produced short felty aerial growth. There was considerable variation in the color of the colonies from different isolates. The predominant color was "Hispano" brown (Plate 14, panel 12-D, Mairz and Paul Dictionary of Color), but some isolates were vinaceous and one was solid black.

Elsinoë ilicis sp. nov.[2]

Laesiones in foliis bigenerae: (A) aliae numerosissimae, parvae, atrae, epiphyllae; (B) aliae pergrandes, usque 1.5 uncias longae, epiphyllae, durae et carbonaceae, typice dimidium laminae occupantes et eam distorquentes; laesiones in caulibus parvae, 0.5–4 mm, planae vel subelevatae, primum brunneae, deinde centro cinerascentes, in baccis parvae, brunneae, planae usque subelevatae, demum suberosae et rimosae; acervuli e mycelio intraepidermicali oriundi, stromatici, per cuticulam erumpentes, 42–112 × 21–42 μ; conidiophora definita non visa; conidia in matrice non visa, in culturis abundantia, hyalina, unicellularia, ovoidea usque ellipsoidea, 3.6–7.0 × 1.5–2.4 μ; ascomata subepidermicalia, irregularia, plerumque parva; asci in stromate irregulariter immersi, pyriformes, 24.0 × 14.0 μ; ascosporae oblongae, hyalinae, septatae, 8.5–12.5 × 3.0–4.0 μ.

In foliis caulibus baccisque Ilicis cornutae, Louisiana.

[2] The writer is indebted to Edith K. Cash for the Latin diagnosis.

Lesions on leaves, stems, and berries. Leaf lesions of two distinct types. Type A, very numerous small black spots on upper surface. Type B, very large lesions, often 1½ inches in length, hard and carbonous, on upper surface, typically on one-half of the leaf blade, causing marked distortion of leaf, occasionally more than one lesion on same leaf. Stem lesions small, ½ to 4 mm, flat to slightly raised, brown at first, later with grayish centers. Lesions on berries small, brown, flat to slightly raised, becoming corky and cracked.

Acervuli arising from intraepidermal mycelium, stromatic, erupting through the cuticle, 42–112 × 21–42 μ; well-defined conidiophores not seen either on host or in culture; conidia (not seen on host but produced abundantly in culture) hyaline, unicellular, ovoid to ellipsoid, 3.6–7 × 1.5–2.4 μ, av. 4.85 × 1.71 μ; ascomata subepidermal, irregular, mostly small; asci imbedded irregularly in stroma, pyriform, 24 × 14 μ; most asci noted empty, few found with mature ascospores; ascospores oblong, hyaline, septate, 8.5–12.5 × 3–4 μ.

On leaves, stems, and berries of *Ilex cornuta* Lindl. Hammond, La. Specimens deposited in Mycological Herbarium, Louisiana State University, Baton Rouge, La. and in Mycol. Collect., Bur. Plant Indus. Soils, and Agr. Engin., also distributed in Jenkins-Bitancourt Myriangiales selecti exsiccati, Fascile 10, No. *471* (paratype) and *472* (type).

INOCULATIONS

A limited number of inoculations were made, using pure cultures of the fungus. While infection was obtained in every case, not all the symptoms as they appear in nature were reproduced. Small potted plants, grown from cuttings, were used in the inoculation tests. All the cuttings came from one tree, which was not diseased although it was growing close to diseased trees. The choice of this tree was probably a mistake, but this was not appreciated at the time. As determined later, the trees exhibited varying degrees of resistance to natural infection, and it would have been better if plant material for inoculation had been taken from trees exhibiting severe symptoms, because this would have indicated that they were susceptible.

In September, 1948, three potted plants with young tender growth were inoculated with isolates obtained from type B lesions. The cultures, which were not sporulating, were macerated in a Waring blender, and the water suspension of mycelial fragments was atomized on the plants. The inoculated plants were kept under high humidity conditions (under bell jars) for five days following the inoculation. Both leaf and shoot infection was obtained. The shoot (bark) lesions were typical of those occurring naturally. The foliage symptoms, on the

other hand, were small black spots somewhat like those of type A. In other words, the large, leaf-distorting lesion (type B) was not reproduced even though the cultures used were isolated from this type of lesion.

In April, 1950, six plants were inoculated. Four of these were inoculated with a mixture of cultures obtained from leaf (type A), shoot, and berry lesions, and two with cultures from type B leaf lesions. All six plants, regardless of the source of inoculum, began to show symptoms about three months after infection. Shoot lesions appeared first as slightly raised purplish spots. The leaves showed very numerous pinpoint black spots. By the end of December the leaf lesions were typical of the small black spot (type A) as it occurs naturally. The shoot lesions had enlarged and developed gray centers. Type B lesions did not develop. The plants were small and not fruiting, so the berry lesions were not reproduced.

SUMMARY

Spot anthracnose, a new and hitherto undescribed disease of Chinese holly (*Ilex cornuta* Lindl.) characterized by lesions on leaves, stems, and berries, is described. The fungus causing the disease is described as a new species under the name *Elsinoë ilicis*.

DEPARTMENT OF BOTANY,
LOUISIANA STATE UNIVERSITY,
BATON ROUGE, LOUISIANA

ADDITIONAL SPECIES OF UREDINALES FROM COLOMBIA [1]

FRANK D. KERN AND H. W. THURSTON, JR.

Studies of the rust-flora of Colombia have been published in papers by Mayor (**15**), Kern and Whetzel (**14**), Kern, Thurston and Whetzel (**13**), Kern and Chardon (**10**), and Kern (**9**). A brief summary of the results presented in the foregoing papers, with some additional notes, is to be found in a paper by Kern and Thurston (**11**). This paper brought the total number of species known from Colombia to that time, 1940, up to 226.

Baxter (**1**) in a recent monograph of the species known to occur on *Salvia* in South America reduces *Uredo Archeriana* K. T. W. (**13**) to synonymy with *Puccinia soledadensis* Mayor (**15**). He also transfers *Uredo Salviarum* Mayor from synonymy with *Puccinia Ballotaeflorae* Long to *Puccinia impedita* Mains and Holw. These changes decrease the total to 224 species. Another change by Baxter affects a name but not the number. This is his reduction of *Puccinia paramensis* Mayor to a variety of *Puccinia conspersa* Diet.

We are now reporting nine additional species, one each in the genera *Dicheirinia* and *Melampsora*, two each in *Puccinia* and *Uromyces*, and three new species, one belonging to the genus *Prospodium* and two that can be assigned only to the form-genus *Uredo*, bringing the total number for Colombia up to 233. We believe that further efforts on the part of collectors will increase this number, as our records show that 292 species have been reported from Venezuela. Several collectors have contributed the specimens here cited and to them we express our thanks for making possible these studies. Their names are included in the citations. We are also indebted to R. A. Toro, G. B. Cummins, and J. A. Stevenson for making certain specimens available to us.

DICHEIRINIA MANAOSENSIS (P. Henn.) Cummins, Mycologia **27**: 158. 1935.

On *Lonchocarpus* sp., Leticia, Colombia, October 15, 1943, Carl O. Grassl *10098* (Ex. Herb. Arthur *F 11081*).

[1] Authorized for publication August 3, 1953 as paper No. 1810 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Contribution from the Department of Botany, The Pennsylvania State University, No. 177.

This is a microcyclic species which is notable for usually having three teliospores united laterally on a common pedicel. Three other species are known, each of them being 2-spored. Cummins, l.c., suggests that "this addition to the 2-spored condition typical of *Dicheirinia* may indicate a tendency toward spore-heads as in Ravenelia."

MELAMPSORA LARICI-POPULINA Kleb. Zeits. f. Pflanzenkrankh. **12**: 43. 1902.

On *Populus nigra* var. *italica* DuRoi (received as *P. deltoides*), La Cruz Nariño, Colombia, October 19, 1943, William C. Davis (Com. J. A. Stevenson).

This collection agrees well with this European species and it seems best to place it here. It does not agree with any of the five North American species of *Melampsora*. Jackson (**7**) has reported a North American species, *Melampsora Medusae* Thüm, from Bolivia.

PUCCINIA FLACCIDA Berk. & Br. Jour. Linn. Soc. **14**: 91. 1873.

On *Echinochloa crus-galli* (L.) Beauv., Medellin, Colombia, August 22, 1946, C. Garcés [*886, 977, 1858*].

These collections were received without host determination. Parts of the inflorescence of the host and the characteristic teliospores of the rust with their oblique or nearly vertical septa, leave no doubt about the reference here made.

PUCCINIA HIERACII (Schum.) Mart. Fl. Mosq. 226. 1817.

On *Hieracium* sp., Piedras Blancos, Colombia, May 6, 1948, C. Garcés [*1629*].

Again this was a collection without host identification as received but with the aid of the inflorescence and the uredospores it was possible to make this determination with assurance.

UROMYCES DOLICHOLI Arth. Bull. Torrey Club **33**: 27. 1906.

On *Cajanus indicus* Spreng. (*Cajan Cajan* Millsp.), Finca Quinta Santander, Rosario, Nte. Santander, Colombia, May 26, 1943, J. E. Orjuela-Navarrete (Herbario Del Laboratorio Fitopathologia No. 1332), II.

UROMYCES STRIATUS Schroet. Abh. Schles. Ges. **48**: 11. 1870.

On *Medicago sativa* L., San Pedro, Colombia, July 25, 1949, C. Garcés [*1754*].

Other names for this species are *Uromyces Medicaginis* Pass. in Thüm. Herb. Myc. Oecon. *156* (1874) and *Uromyces striatus medicaginis* (Pass.) Arthur, Manual of the Rusts in U. S. and Canada, P. 299 (1934). Jackson (**8**) reported it under the name *U. Medicaginis* from Argentina with the comment that it was known from South America only from Argentina and southern Brazil. It is common in North America and Europe.

Prospodium Garcesii sp. nov.

Pycniis et aeciis ignotis.

Uredosoris hypophyllis, plerumque in maculis decoloratis dense aggregatis, cinnamomeo-bruneis, per stomata erumpentibus, cyathiformibus, 60–125μ diam.; peridio pallide flavescenti; paraphysibus peripheric is, incurvatis, peridio concolori, cylindraceis, plerumque ad apicem acuminatis, 9–12 × 32–48μ; tunica ca. 1.5μ. cr. saepe ad apicem convexa; lateribus 3μ cr.; uredosporis globosis vel late ellipsodeis, 18–23 × 23–27μ; tunica pallide cinnamomeo-brunea vel flavescenti, ca. 1.5μ prominenter echinulata; poris 2, equatorialibus.

Teliis ignotis.

On *Arrabidaea magnifica* Sprague, Fac. de Agronomía, Medellin, Colombia, September 29, 1942, C. Garcés *538;* Robledo, Colombia, November 24, 1945, C. Garcés [*152*].

In an excellent paper on the genus *Prospodium,* published in 1937, Dr. G. B. Cummins (**3**) adequately described the extrostomatal development of the uredinia and telia in this genus, which had been understood imperfectly by previous investigators. In his monograph of this genus in 1940, Cummins (**4**) established the Section *Cyathopsora* on the basis of the extrastomatal character and included fourteen species in the group. We (**12**) described an additional one, *Prospodium Cumminsii,* and are now adding another with confidence even though known only in the uredo-stage.

Uredo Diplostephii sp. nov.

Uredosoris hypophyllis, sparsis, in maculis decoloratis dispositis, rotundatis, 0.3–0.5 mm diam., flavis, pulverulentis epidermidi rupta inconspicua; uredosporis globosis, ellipsoideis, vel obovoideis, 23–31 × 30–39μ; tunica hyalina crassitudinis inconstantis, 2.5–3μ, quibusdam areis incrassatis, 4–4.5μ, moderate echinulata; poris obscuris.

On *Diplostephium eriophorum* Wedd., Paramo del Ruiz (Caldas), Colombia, May 1940, J. Cuatrecasas *367, 693* (type).

A characteristic of this species is the variable thickness of the walls, i.e., the presence of irregular thicker areas. In that regard it resembles *Uredo Olearíae* Cooke (**2**) on the genus *Olearia,* but it differs in the size of the spores and in the range of the thickness of the walls. *Uredo*

Oleariae is an endemic species from the Auckland Islands. According to the phanerogamic treatises the genera *Diplostephium* and *Olearia* are closely related and that they should have similar, but not identical rusts, could be expected. *U. Oleariae* is well described, and illustrated, by Cunningham (**6**).

Uredo diocleicola sp. nov.

Uredosoris amphigenis, sparsis, vel in greges minutos dispositis, punctiformibus, rotundatis vel ovatis, 0.2–0.3 mm diam. pulverulentibus, cinnamomeo-bruneis; epidermidi rupta et conspicua; uredosporis late ellipsoideis vel obovoideis, 16–24 × 22–32μ; tunica cinnamomeo-brunea, 1.5μ cr., minute echinulata; poris 2, equatorialibus.

On *Dioclea columbiana* Killip. Medellin, Colombia, November, 1941, C. Garces Orjuela and Royas Pena (Herbario del Laboratorio Fitopathologia No. *967*).

This species differs from *Uredo Diocleae*, described by Cummins (**5**) from Sierra Leone, in having somewhat larger spores, with finer echinulations, showing no tendency to asymmetrical or reniform shape.

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THE SECTION GENEVENSIS OF THE GENUS MUCOR

C. W. HESSELTINE[1, 2]

(WITH 1 FIGURE)

Traditionally species of the genus *Mucor* have been variously grouped into subgenera or sections. To clarify the taxonomic chaos into which the members of the genus have fallen it seems best to continue to place groups of species into sections. The species in the sections have been grouped for convenience rather than to show any phylogenetic relationships, although in many cases the sections do represent such a natural assemblage of species.

Schroeter (**9**) reduced certain of van Tieghem's genera to the subgenera *Eumucor, Circinella, Rhizopus* and *Spinellus* within the genus *Mucor*. The last three are now considered as distinct genera. Fischer (**3**) in 1892 considered that the four subgenera created by Schroeter actually deserved generic status and divided the species remaining in *Mucor* into three main groups based upon the mode of branching of the sporangiophores. Lendner (**7**) later, in 1908, divided the species similarly into three sections, *Monomucor, Racemomucor* and *Cymomucor*, each of which was characterized by the particular system of branching.

In his treatment of the genus *Mucor*, Zycha (**10**) rejected this system of grouping species and adopted sections based upon the shape of the sporangiospores, the height of the sporangiophores, and the color of the cultures. Sections were designated as *Sphaerosporus, Ramannianus, Racemosus, Fragilis, Hiemalis, Flavus* and *Mucedo*. *Sphaerosporus* included those species with spherical sporangiospores. *Ramannianus* was characterized by the single species *M. ramannianus* with its very short sporangiophores. The members of the section *Racemosus* possessed chlamydospores in the sporangiophores and had short oval sporangiospores as contrasted with the section *Fragilis* with chlamydospores mostly

[1] Contribution from the Northern Regional Research Laboratory, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Peoria, Illinois.

[2] Part of this material is taken from the unpublished thesis of the author submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Wisconsin.

missing and with the sporangiospores approximately twice as long as broad. These two sections were separated from *Hiemalis* by producing gray or brown-colored colonies, strongly branched sporangiophores and the sporangial wall breaking or only slowly deliquescing. The sections *Flavus* and *Mucedo* were different from all the other sections in being larger forms. Section *Flavus* had sympodially divided sporangiophores while the section *Mucedo* was never sympodially branched. This system is the one used by Christenberry (**2**) in one of the few treatments of the genus in America.

In 1939 Naumov (**8**) went further than previous workers, breaking the genus into thirteen sections. Each of these was given a new section name created by Naumov. They are as follows: *Micromucor, Circinellastrum, Lendnerella, Heteropus, Bonordenia, Hagemia, Byssomucor, Fischerella, Macromucor, Thamnidioides, Piromyces, Absidioides* and *Rhizopoides*. Unfortunately Naumov does not give a key to the sections although keys are given to the species in each section.

The proposed section *Genevensis* is one of the most readily recognized and best defined sections of the genus *Mucor*. The following key is given for the separation of the major groups into which *Mucor* was divided in Hesseltine's (**5**) treatment of the genus.

1. Colonies flat, velvety, reddish or ashy gray in color, not over 3 mm in height; sporangiospores less than 4 μ in length.....Section *Ramannianus*.
1. With not all the characters above.....2.
2. Sporangiospores spherical, occasionally a few very short oval spores may be present with the spherical ones.....Section *Sphaerosporus*.
2. Sporangiospores not spherical or at least only a few spherical.....3.
3. Homothallic species, that is, with zygospores always present..Section *Genevensis*.
3. Heterothallic species, zygospores not formed in single-spore isolates.....4.
4. Chlamydospores present in the aerial mycelium in large numbers Section *Racemosus*.
4. Chlamydospores absent or in the substrate mycelium, or, if present, in the aerial mycelium, only in old cultures and uncommon.....5.
5. Species with sporangia averaging over 100 μ in diameter or with sporangia definitely at two levels.....6.
5. Species with sporangia averaging less than 100 μ in diameter.....8.
6. Sporangia over 500 μ in diameter with sporangiophores usually up to 10 cm in height and 50–100 μ in diameter.....Section *Macromucor*.
6. Sporangia less than 400 μ in diameter.....7.
7. Colonies with tall, erect sporangiophores usually unbranched above, with a second short fertile region of smaller sporangiophores and smaller sporangia near the surface of the substrate, these short sporangiophores repeatedly branched Section *Mucedo*.
7. Sporangiophores simple or usually only once or twice branched; colonies without an abundant region of fruiting near the substrate.....Section *Flavus*.

8. Mycelium at first white, later gray or brown; sporangiophores strongly branched; sporangial wall persistent or only slowly deliquescing.....Section *Fragilis.*
8. Mycelium white, later yellowish or light gray; sporangia rapidly deliquescing; not strongly branched.....Section *Hiemalis.*

Section **Genevensis** n. sect.

Section containing *Mucor* species which are homothallic.

Parte continente species Mucoris quae sunt homothallicae.

In the family Mucoraceae the homothallic species of the *Genevensis* section are distinct from the homothallic genus *Zygorhynchus* by the morphology of the zygospore. In this section of *Mucor* the zygospores have both suspensors straight and essentially equal in diameter and length. Perhaps an exception to this is the species *Mucor parvispora* where some zygospores are formed between somewhat unlike suspensors. In *Zygorhynchus,* on the contrary, the zygospores are borne between one long, curved suspensor that is typically very large in diameter and a short straight one that is much smaller in diameter than its mate. The type species of the section is *Mucor genevensis* Lendner.

A search of the literature indicated that two and perhaps three species of *Mucor* belong in this section. The following key is offered for purposes of separating these species and the new species which is herein described.

1. Sporangiospores very minute, bacterium-like, 3–5.5 × 1.25 μ in size *Mucor bacilliformis.*
1. Sporangiospores with a greater width, the width to length ratio not so great...2.
2. Colonies less than 1 cm in height.....*Mucor philippovi.*
2. Colonies at least 1.5 cm in height.....3.
3. Sporangiospores spherical, oval or even elliptical in shape in the larger ones, never twice as long as broad, 4–6.5 × 2.5–3.5 μ.....*Mucor parvispora.*
3. Soprangiospores typically elliptical, often twice as long as broad, averaging 8 × 4 μ.....*Mucor genevensis.*

Mucor bacilliformis sp. nova

Sporangiophoris non ramosis, serius tarde sympodialiter ramosis, 5–15 μ diameter; sporangiis globosis, fulvis, 30–50 μ diameter; tunicis sporangiorum planis, liquentibus; columellis globosis vel oviformibus, collari male descripto ad nullo, ad 26 × 21 μ; sporangiosporis mensura aequalibus, bacteriis similibus, oblongis, fusiformibus, reniformibus, aut in uno latere complanatis, constanter cum granulo atro in utro extremo, 3–5.5 × 1.25 μ; zygosporis fere globosis, brunneis vel paene brunneolonigris, 35–72 μ, mycelio homothallico.

Colonies pallid quaker drab (Ridg.), margin irregular, with sporangial colonies, reverse white, then cream, finally grayish olive (Ridg.) in

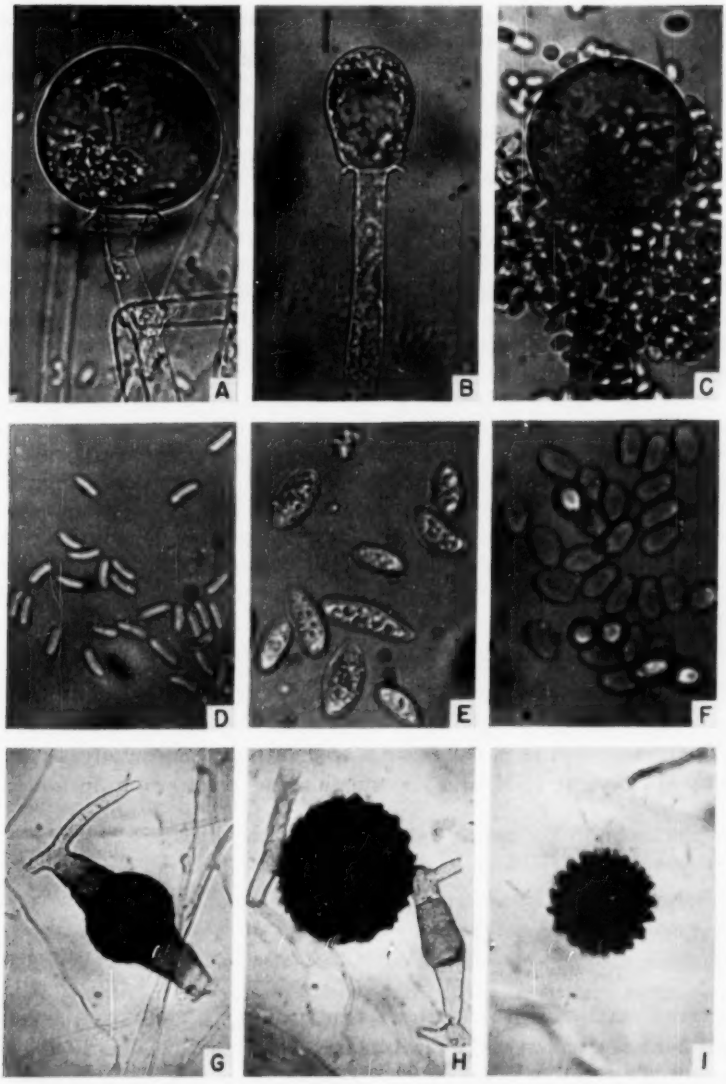

FIG. 1. a. Columella of *Mucor bacilliformis*, × 825; b. Columella of *Mucor genevensis*, × 825; c. Columella of *Mucor parvispora*, × 825; d. Sporangiospores of *Mucor bacilliformis*, × 1325; e. Sporangiospores of *Mucor genevensis*, × 1325; f. Sporangiospores of *Mucor parvispora*, × 1325; g. A zygospore of *Mucor bacilliformis*, × 262; h. A zygospore of *Mucor genevensis*, × 262; i. A zygospore of *Mucor parvispora*, × 262.

center (growing down as much as 1 cm into the agar in a slant), up to 1.5 cm in height above the substrate; odor faint; sporangiophores unbranched, later tardily sympodially branched, 5–15 μ in diameter, with septations above the origin of the branches, often constricted just below the sporangium; sporangia spherical, pale yellowish tan, moist, 30–50 μ in diameter; sporangial wall smooth, deliquescing rapidly; columellae globose to ovoid, when ovoid usually larger, hyaline, collar poorly defined to none, globose ones 10–25 μ in diameter, averaging 15–17 μ, short ovoid ones up to 26.6 × 21 μ (FIG. 1, a); sporangiospores uniform in size and shape, very small, bacterium-like, elliptical, fusiform, reniform or flattened on one side, regularly with a dark granule at each end, 3–5.5 × 1.25 μ (FIG. 1, d); giant cells in substrate filled with oil droplets, intercalary, up to 60 μ in diameter; chlamydospores intercalary, thick-walled, same diameter as mycelium, granular; oidia globose, at ends of branches in substrate, slightly yellowish, granular, when freed budding as yeast, up to 35 μ in diameter; zygospores borne near the substrate upon zygophores, nearly spherical, brown to brownish black, containing several large oil droplets, 35–72 μ in diameter (FIG. 1, g); exospore irregularly roughened, surface covered by a number of clumps of bands which darken in age as the projections of the zygospore, homothallic; suspensors brownish, formed often just below the sterile end of a zygophore, straight, equal or nearly so, contents granular, enlarging toward the zygospore.

This species was found but once from a soil sample collected by Dr. E. M. Gilbert near Hayward, Wisconsin, September 1940. The sample consisted of sandy soil obtained at eight inches below the surface.

The description is based upon a seventeen-day-old culture on Synthetic Mucor agar (SMA).[3] Sporulation and zygospore formation also

Dextrose	40	gm
Asparagine	2	gm
KH_2PO_4	0.5	gm
$MgSO_4$	0.25	gm
Thiamine chloride	0.5	mg
Agar	15.0	gm
Distilled water	1000.0	ml

occurred readily upon soil extract agar, malt agar, and potato-dextrose agar but failed on hay and Czapek agar. The absence of almost all aerial mycelium but the presence of giant cells on these last media is striking. On SMA, colonies at seventeen days are 7.5 mm in diameter with the sporangia resting against the lid of the Petri dish. This culture had been carried for several years before being critically studied and in all

[3] Synthetic Mucor agar is prepared as follows:

the transfers the zygospores were formed in abundance. Later transfers were made from single sporangia and in each case these yielded cultures which produced zygospores. As a final check, dilution plates were poured and single colonies were isolated which appeared to arise from single sporangiospores. Each of these isolates was found to produce zygospores in abundance.

The species is easily distinguished, aside from being homothallic, by the smooth outline of the zygospore, by its ability to grow deeply into the medium and by its bacterium-like sporangiospores. This later character is the one upon which the specific name *bacilliformis* is based.

The type culture is maintained at the Northern Regional Research Laboratory, Peoria, Illinois, as NRRL *2346* and has been deposited at the Centraalbureau voor Schimmelculture, Baarn, Holland. Herbarium material has been deposited in the Herbarium of the University of Wisconsin.

MUCOR PHILIPPOVI Naumov, Clés des Mucorinées, p. 44. 1939.

Colonies uncolored, without sterile aerial mycelium, 6–8 mm in height; sporangiophores rarely upright and straight, more often bent and prostrate, phototrophic, rarely branching, usually with no more than one branch, 8–11 μ in diameter; sporangia uncolored, hygroscopic, moist, 90 μ in diameter; columellae spherical, more often elongate, often with granular contents, 25–38 × 19–33 μ; sporangiospores narrowly ellipsoidal, nearly fusiform, 6–9.6 × 2.7–5.5 μ, but sometimes spherical or reniform; zygospores 50–100 μ, dark brown, with radiate protuberances up to 7 μ in width; zygophores 20–25 μ wide, homothallic.

Source of culture not given by Naumov.

The above diagnosis was prepared from Naumov's description as it appears in his key. The colonies of this fungus were said to be less than one half as high as that of *Mucor genevensis*. *Mucor philippovi* may eventually prove to be only a low-growing variety of *Mucor genevensis* or a form which *Mucor genevensis* assumes on certain unfavorable media. So far as the author is aware no culture or herbarium material of this organism is available for study.

MUCOR PARVISPORA Kanouse, Pap. Mich. Acad. Sci. **3**: 123. Pls. 28–29. 1924.

Colonies less than 3 cm high, white, becoming drab-gray to smoky gray (Ridg.); sporangiophores branched sympodially, each branch cut off by a cross-wall, hyaline, smooth, bearing sporangia terminally at end

of branches, up to 20 μ in diameter; sporangia spherical, 20–60 μ in diameter; sporangial wall smooth, translucent, allowing spores to be clearly seen inside, fragile, when broken leaving a small collar; columellae mainly spherical, sometimes oval, 20–48 μ in diameter (FIG. 1, c); sporangiospores small, hyaline, smaller ones ovoid to spherical, larger ones ellipsoidal, 4–6.5 × 2.5–3.5 μ (FIG. 1, f); chlamydospores in the vegetative mycelium or substrate, single or in chains up to 5 or 6, ellipsoidal, thick-walled; oidiospores also sometimes present; zygospores formed between somewhat unequal suspensors, spherical to slightly flattened, golden brown, covered with pointed, warty protuberances, 20 × 18 μ to 65 × 65 μ, a few up to 90 μ, homothallic (FIG. 1, i); azygospores present.

Isolated from fresh horse dung, Ann Arbor, Michigan.

The above data were taken from the description given by Kanouse which was prepared from a culture grown upon fresh white bread. Additional data are drawn from an examination of part of the type specimen. This species is closely related to *Mucor genevensis* but differs from it in the shape and size of the sporangiospores and in the dimensions of the sporangia and zygospores. The sporangiospores of *Mucor parvispora* are smaller and are mostly ovoid to globose while those of *Mucor genevensis* are more elongate. Sporangia and zygospores are smaller than in the latter species. The brilliant golden brown of the zygospore is quite pronounced.

MUCOR GENEVENSIS Lendner, Les Mucorinées de la Suisse, p. 80. Fig. 27. 1908. Also Bull. Herb. Boissier **8**: 79. 1908.

? 1884. *Mucor erectus* Bainier, Ann. Sci. Nat. VI. **19**: 207.

? 1902. *Mucor alpinus* Hansen, Compt. Rend. Carlsberg **5**: 96.

Colonies white, actually pale smoke-gray (Ridg.), up to 1.5 cm in height, with sporangial colonies; reverse white, tinted with yellow; odor strong, *Mucor*-like; sporangiophores hyaline, branched sympodially, with a cross-wall above the origin of each branch, over 1 cm in height, to a width of 18 μ; sporangia at first white, then with a light yellow tint, glistening, rapidly deliquescing, spherical to slightly flattened, up to 100 μ in diameter, usually 40–90 μ; columellae hyaline, pyriform to ovoid, mostly pyriform, smaller ones almost cylindrical, up to 36 × 48 μ (FIG. 1, b); sporangiospores hyaline, ovoid to ellipsoidal, sometimes flattened on one side, slightly yellowish in mass, containing a few scattered granules, largest spores 12.4 × 6 μ, smallest 5 × 2.5 μ, average 8 × 4 μ (FIG. 1, e); zygospores always numerous, produced near the substrate, giving the culture a blackish zone, spherical to slightly compressed, with heavy spines up to 4.2 μ in length, at first brown then blackish brown, with oil droplets inside, formed between sporangiophores, 30–100 μ in diameter

(FIG. 1, h); suspensors straight, unadorned, equal or slightly unequal; oidia abundant in submerged mycelium, spherical to ovoid, with a heavy clear wall, filled with granular protoplasm, single or in a series at the end of branches, when in a series the terminal spores smaller, up to 39 μ in diameter, later breaking off and undergoing budding.

The material studied consisted of two cultures obtained from the Department of Bacteriology of the University of Wisconsin, several isolates from Wisconsin soils, one from Swedish soil, and NRRL isolates *1407, 1408, 1409, 1410, 1411, 1412* and *1758*.

The above description was prepared from a study of eight-day-old cultures on SMA. At this age the colonies had not yet covered the surface of the agar in a Petri dish but had fruited against its lid. One of the characteristic attributes of these cultures is the mature sporangia which bend over and touch the agar beyond the margin of the colony. Wherever such a sporangium comes into contact with the substrate the sporangiospores germinate in place to give rise to small colonies. These colonies are observed about the whole margin of the old colony. Many zygospores are formed upon soil extract agar.

The description given above was based upon cultures NRRL *1755, 1756,* and *1821*. Culture NRRL *1821* represented a typical soil isolate from Wisconsin which had been isolated from hardwood forest soil collected near Mount Vernon, Wisconsin. Cultures *1755* and *1756* were obtained from the Department of Bacteriology which had received them originally from Dr. Blakeslee in June 1932.

Isolate NRRL *1407* appears to be an entirely typical representative of this species. NRRL *1408* and *1409* are mutants; the latter culture on SMA produces a mealy or bacterium-like growth without zygospores or sporangia.

Mucor genevensis is repeatedly isolated from soil samples.

Whether *Mucor genevensis* represents the correct name of this homothallic species is uncertain. Lendner stated that it was very similar to *Mucor erectus* Bainier 1884 except that the zygospores are borne upon special zygophores and not between sporangiophores as is indicated for *Mucor erectus*. In addition the sporangia are smaller in size. Authentic cultures of this species are not known to exist.

Zycha reported that a culture obtained from the Baarn collection representing Hansen's *Mucor alpinus* 1902 (**4**) was actually only *Mucor genevensis*. The description given by Hansen is unsatisfactory and from it it is impossible to know exactly what he was working with. It, therefore, seems proper to accept *Mucor genevensis* as the valid name for the species. *Mucor alpinus* Hansen and *Mucor erectus* Bainier are

probably both synonyms. However, since both these species were so poorly described and since they were described prior to the discovery of the significance of homothallism it is better to retain the epithet *genevensis* for which a very adequate description was given.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Drs. E. M. Gilbert and M. P. Backus of the University of Wisconsin, and Miss Dorothy I. Fennell and Dr. R. G. Benedict of the Northern Regional Research Laboratory.

NORTHERN REGIONAL RESEARCH LABORATORY,
PEORIA, ILLINOIS

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AN INDEX TO C. L. SHEAR'S MYCOLOGICAL NOTES I–IX

EDITH K. CASH

Under the title "Mycological Notes," Dr. C. L. Shear published a series of nine papers in volumes **29–40** of MYCOLOGIA (1937–1948). The numerous genera and species of Ascomycetes and Fungi Imperfecti discussed in these articles are here indexed with a view to facilitating their consultation by mycologists; the volume, pages, and date cited refer in each case to MYCOLOGIA.[1]

[1] I wish to express my gratitude to Miss Cash for preparing this index to my Notes. I hope it will make them more readily accessible to anyone who cares to consult them.—C. L. Shear.

U. S. PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

WILLIAM CHAMBERS COKER

JOHN N. COUCH [1] AND VELMA D. MATTHEWS [2]

(WITH PORTRAIT)

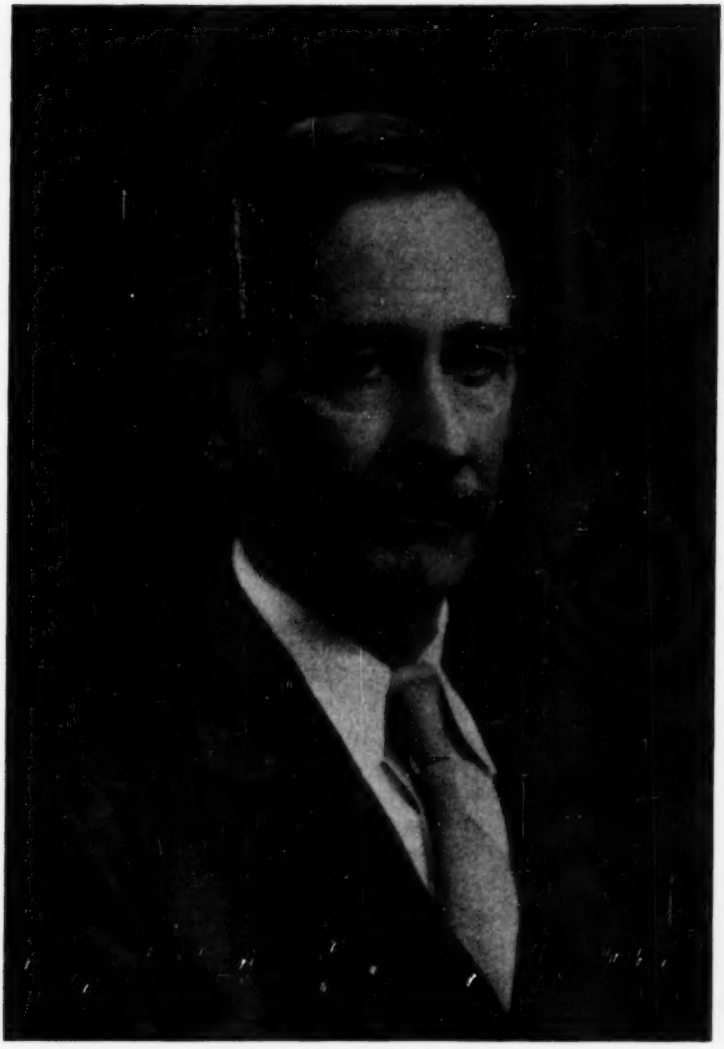
In the death of Dr. W. C. Coker on June 27, 1953, botanical science, particularly mycology, has lost one of its most outstanding men. So numerous were his contributions and so great was his influence that some account of his life, his work and his personal qualities seems fitting here.

William Chambers Coker was born on October 24, 1872, in Hartsville, South Carolina. His father, Major James Lide Coker, born at nearby Society Hill, was educated at the Citadel in Charleston and spent one year at Harvard doing practical analytical work in chemistry and working with small groups under Asa Gray and Louis Agassiz. The Major, one of South Carolina's most successful and farsighted businessmen, was also a leader in education, as shown by his help in the establishment in South Carolina of the public school system, a private high school, and Coker College for women. Dr. Coker's mother, Susan Armstrong Stout, was the daughter of the Reverend Platt Stout, a Baptist minister of Carlowville, Alabama. He was named for his mother's uncle, William Chambers.

At an early age, Dr. Coker's interest in nature was shared and encouraged by his father. The Major took the older boys for walks in the woods and fields on Sunday afternoons, instructing them in natural history; and he permitted them to use the books, microscope, and considerable apparatus and materials which he had brought back from Harvard for conducting experiments in agricultural chemistry.

During those days much of the teaching of the children took place in the home. In an unpublished manuscript, "Childhood Recollections of My Father," Dr. Coker relates an interesting incident of this period: "We had at one time when I was in my early teens a . . . governess from Virginia . . . to whom I took a strong dislike which became so bad that I refused to study and did not answer her questions. This was reported to father and he called me in and asked me what I would rather do, attend to my studies or go to work on the farm. He said that if I

[1] University of North Carolina, Chapel Hill, North Carolina.
[2] Coker College, Hartsville, South Carolina.



WILLIAM CHAMBERS COKER

decided to work on the farm, to report next morning to his farm superintendent at six o'clock. I said nothing but reported for studying the next morning and behaved myself afterwards." Later, Dr. Coker attended a small college preparatory school in Hartsville.

In 1891, he entered the University of South Carolina. Among his teachers were Professor James Woodrow, Ph.D. (Heidelberg), M.D., D.D., LL.D., who held the chairs of biology, geology and mineralogy, and W. B. Burney, Ph.D. (Heidelberg), who held the chair of chemistry. It is interesting to note that at his death Dr. Coker left to the University of South Carolina Library a gift honoring James Woodrow. He tells us that his father gave him no instructions as to what courses to take or how to behave, and no advice on joining a fraternity or whether to go into athletics. However, he played tennis on the varsity team, and took an active part in the Euphradian Literary Society. He states that, upon his graduation in 1894, when Major Coker noted from the program that his son was a "Highly Distinguished" graduate, "He immediately expressed his appreciation and for the first time, I think, in my life he put his arm around my shoulder and said: 'This is gratifying.'"

The year of his graduation he began working in the Atlantic National Bank at Wilmington, North Carolina, becoming one of the vice-presidents two years later, in 1896. The next year, he decided to give up banking and to do graduate work in botany at the Johns Hopkins University. In this decision he received the whole-hearted support and encouragement of his family.

At Johns Hopkins, the untimely death of Professor James Ellis Humphrey had left his student, Duncan S. Johnson, as the only botanist in the biology department. Johnson received his Ph.D. in 1897 and took over the work of Humphrey. Dr. Coker, entering in the fall of 1897, was Johnson's first student. Dr. Johnson gave only one formal course; this ran from September to June and was planned to cover the whole range of plant morphology and related fields. After completing this very thorough and difficult course and certain required courses in zoology, a graduate student was ready to start on his research. Dr. Coker's dissertation, "On the Gametophytes and Embryo of *Taxodium*," was completed in 1901, and the degree of Ph.D. was awarded at the June commencement. This paper, a classic in seed development, was published in the *Botanical Gazette* as *Contribution No. 1* from the Botanical Laboratory of Johns Hopkins University. At that time it was customary for new Ph.D.'s to spend some time studying in Europe. Accordingly, Dr. Coker spent a semester, 1901–1902, in Strasburger's laboratory at Bonn-am-Rhine, Germany.

In the fall of 1902, Dr. Coker joined the faculty of the University of North Carolina at Chapel Hill as associate professor of botany, and served there continuously, until his retirement in 1945 as Kenan Research Professor of Botany, Emeritus. Although he received offers to

go elsewhere, it seems that he never seriously considered any of them.

During a period of about fifty-one years, one hundred and thirty-seven publications appeared. Seven of these were books and the others ranged from long monographs to abstracts over a very wide field of interests. Although his dissertation was followed by several short papers of a similar morphological nature, Dr. Coker's main botanical work developed in other directions.

In the Chapel Hill area, Dr. Coker found a region exceedingly rich botanically but largely unexplored. Curtis had worked in the Hillsboro area fourteen miles away, but his large manuscript on the higher fungi was never published and his "Woody Plants of North Carolina" had long been out of print. Dr. Coker immediately began work on the local flora and published within a year a list of the woody plants of Chapel Hill. Interest in the woody plants was continued and the work was expanded with the help of H. R. Totten to include first the "Trees of North Carolina," published in 1916, and later extended to the "Trees of the Southeastern States," published in 1934. The third edition of this book appeared in 1945, the year of Dr. Coker's retirement from active service in the department. A partially completed work on the shrubs illustrated with many beautiful paintings was left at his death. He was not satisfied in merely knowing the native plants but was concerned also with their uses, including their suitability for use in landscape design. This practical interest resulted in the publication of two bulletins on landscape design in the early 'twenties, and the planting of shrubs, under his direction, on many of the school grounds throughout the state. Other important publications by Dr. Coker were "Vegetation of the Bahama Islands" (1905) and "The Plant Life of Hartsville, S. C." (1912). In its largely ecological treatment, the latter was significantly ahead of its time. His papers on science teaching in the high schools, the Venus' fly-trap, algae, bryophytes, and ferns, and several exceedingly interesting sketches of the lives of southern botanists, show the great diversity of his interests and the breadth of his training. As a botanist, however, he will certainly be remembered longest for his researches on fungi.

Dr. Coker arrived in Chapel Hill in the fall of 1902 with a copy of Atkinson's "Mushrooms" and, from the records of his collections that appear on the margins of numerous pages, it is evident that he began using it almost immediately. No specimens of mushrooms were kept until 1908, when Davie Hall was built and botany established as a separate department with space for an herbarium. In 1917 his monograph of the Amanitas of the Eastern United States appeared, followed in quick succession by monographs on other groups of the white-spored agarics.

Meanwhile, studies were being made on all the groups of higher basidiomycetes as they were collected. When this work was in full progress, in the late 'teens and 'twenties, the various manuscripts were kept in manila folders on open tables in the center of the research laboratory and were weighted down by boards. On a few occasions a careless assistant forgot to replace one of the boards and cross drafts scattered the manuscripts, drawings and photographs all over the laboratory and sometimes even out on the campus.

At this time, intensive work was in progress on the lower Basidiomycetes, Thelephoraceae, Clavariaceae, Boletaceae, Hydnaceae, and the Gasteromycetes, resulting in the publication of long papers in the Mitchell Journal on the first two, and of four large books on the latter groups, the last of which appeared in 1951 when Dr. Coker was seventy-nine years old. In addition, numerous shorter papers on the higher fungi were published during this period, and several long manuscripts on the Polyporaceae and the pink-, purple-, cinnamon-, and black-spored agarics were left unfinished at his death.

In spite of his prodigious researches on the higher fungi, and the high quality of these as taxonomic contributions, Dr. Coker's fame as a mycologist rests largely on his work on the Saprolegniaceae, begun in 1908 and reaching its climax in the publication of "The Saprolegniaceae" in 1923, a work which has had world-wide influence in stimulating researches on the aquatic fungi.

Dr. Coker's eminence as a mycologist and botanist brought him honors at home and abroad. In 1927 he was elected chairman of the Mycological Section of the Botanical Society of America, and in 1935 he presented an invitation paper on the aquatic fungi before the Mycological Section of the Sixth International Plant Congress held in Amsterdam, Holland. In 1950 he was appointed vice-president of the Mycological Section of the Seventh International Plant Congress at Stockholm, Sweden, but was obliged to decline the honor because of failing health. He was the first chairman of the Southeastern Section of the Botanical Society of America, serving from January, 1939, to December, 1941. The many other local offices and honors which came to him are too numerous to mention here.

Fortunately, Dr. Coker realized the primary need for building up a good library and herbarium. Starting with a very meager botanical library he succeeded in acquiring many of the old standard serial journals in complete sets, sometimes paying for them himself when University funds were not available. Under his direction a good working herbarium particularly rich in the higher fungi and the woody plants of the southeast has been accumulated.

The achievements of Dr. Coker most easily seen and appreciated by non-botanists were in landscape architecture. In this he had a remarkable talent constantly improved by study and practice. Soon after he arrived in Chapel Hill he obtained permission of the University authorities to develop about six acres of boggy waste land on what was then the eastern edge of the campus. This unsightly area was gradually transformed by him into a natural garden featuring mostly native shrubs and trees. For thirty years he was chairman and the "guiding light" of the Building and Grounds Committee. His good judgment and taste are reflected in dignified, uncrowded buildings softened by informal plantings. With unusual foresight he realized that the trees on the campus would not live for ever, and directed the planting of many young ones. A considerable part of his home garden was used for the propagation of shrubs and ornamentals which for many years were given away to the people of Chapel Hill along with advice or even the personal supervision of their planting. For several years, Dr. Coker carried on some experiments with grasses in an attempt to find a suitable mixture of seed which would keep the lawns of Chapel Hill green throughout the year. Finally a mixture was selected and a pamphlet was published telling in great detail how to prepare and fertilize the ground and plant the seed. The directions for mowing were simple: cut what comes!

For many years, Dr. Coker's major interests in teaching were devoted to the course in general botany, which seems to have been modelled much after Dr. Johnson's at Hopkins. As an undergraduate teacher he ranked high among the faculty, although his methods were sometimes not wholly conventional. In his general botany classes he began with the low forms of plant life and usually progressed to the more advanced types; the exact procedure, however, was never twice the same. A walk from his home through the village and Arboretum might suggest an entirely new topic for that day's discussion, and the assistant who had prepared the classroom demonstrations could never foretell when a complete change might have to be made in the last few minutes before the lecture began. But the lecture fitted the time and caught the student's interest, and so often contained fresh observations on living plants and was so graphically presented that the student was encouraged to find out something new about plants for himself. The excellence of the laboratories was due mainly to Dr. Coker's insistence that the students have plenty of fresh and interesting plant material with which to work, and his skillful use of field trips to supplement the lecture and laboratory.

As a teacher of graduate students and director of research he was superb. In the advanced classes there were rarely over three or four

students, each of whom had a table in the research laboratory along with the professor. Such small classes were conducted very informally, the students themselves doing the lecturing by giving reports of research papers which were related to the work being done in the laboratory. Dr. Coker was never too hurried or too busy to look at something of interest which a student had found. To a remarkable degree, he had the rare ability to stimulate the student to want to find out more for himself and to believe in the importance of what he was doing, if it added to knowledge. It is not difficult to explain in part this inspirational power, for Dr. Coker regarded his own work as of tremendous importance and, until his health began to fail, devoted himself almost entirely to it. In fact, one of his most distinctive characteristics was his single-minded devotion to his work.

For over fifty years, Dr. Coker was one of the most striking figures in Chapel Hill. He was tall and slender and had unusual personal charm. He was in every sense a gentleman—dignified but friendly, calm and reserved but sensitive to the needs of others, often quietly opening up avenues of financial assistance to struggling students. He helped many young men through college by making them personal loans. Though he lost money on some, he never lost faith in people. He was not talkative, but in subjects such as natural history, in which he was interested and well read, he was a most engaging conversationalist. He was fond of animals, and although he always chose a mongrel for his dog, the dog somehow assumed the aristocratic dignity of its master.

Dr. Coker remained a bachelor until October 28, 1934, when he married Louise Manning Venable, daughter of President Venable, and her companionship and affection filled the one void in his life. Their home became a mecca for neighbors, friends, and a multitude of devoted nephews and nieces. Their talk and laughter amused and delighted the genial host, crowning a full and fruitful life with a joyous old age. Finally, as his strength ebbed, so serene were his surroundings and so gentle his care, that his span of life was stretched to four score years.

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NOTES AND BRIEF ARTICLES

A TECHNIQUE FOR THE ASEPTIC REMOVAL OF CONIDIA FROM AGAR CULTURES

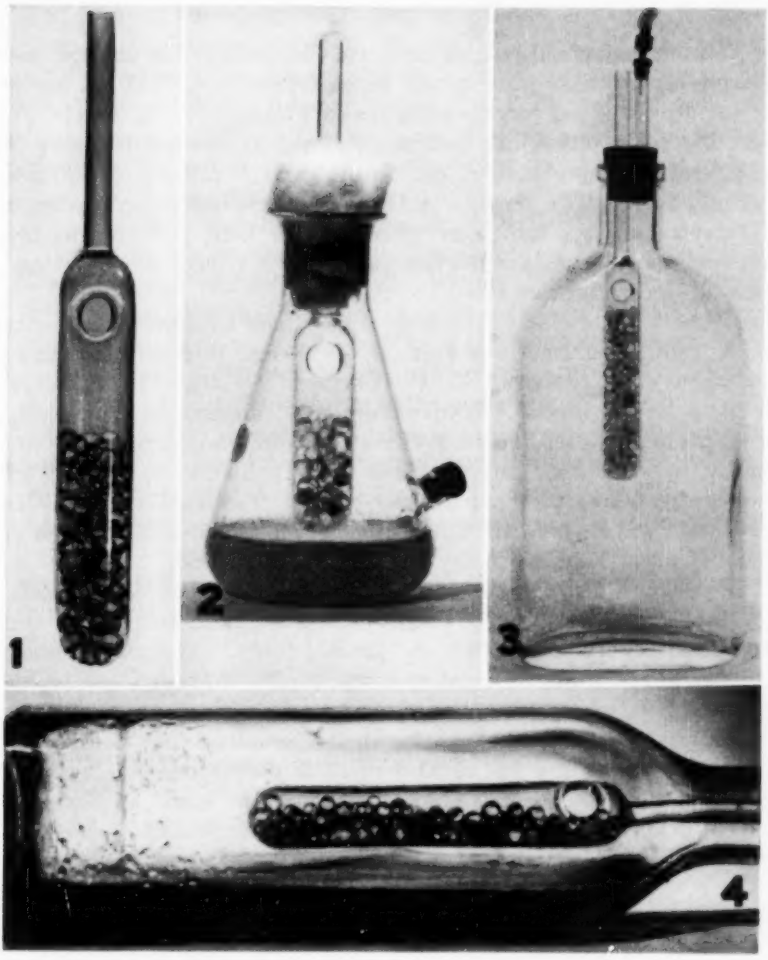
A number of fungi are characterized in part by possessing conidia which do not readily separate from the conidiophores. This is particularly true with some members of the Moniliales. A method of forcibly dislodging these conidia is required where aseptic handling is of prime importance. The use of a flame-sterilized inoculating loop or needle to scrape the sporulating surface is impractical when cultures are propagated in large flasks, and the probability of contamination in any size flask increases with repeated removal of the plug.

Arising out of the necessity for aseptic removal of fungus spores for use in physiological studies, a glass bead container (FIG. 1) was devised. The glass beads substitute for needle-scraping the sporulating surface. The pellet container consists of a glass tube with a diameter slightly less than that of the mouth of the culture vessel. A small hole is blown into the end of the tube nearest the flask stopper to allow the glass beads to drop out when the flask is tilted. A glass rod "handle" positions the container above the medium in the flask (FIGS. 2, 4), and is inserted into a rubber stopper (FIG. 3) or cotton plug (FIG. 2).

A small glass tube stoppered with a serum bottle cap is inserted in the rubber stopper (FIG. 3). Inoculum, and sterile water or broth media, may be injected through this inoculating tube with a sterilized hypodermic syringe and needle. In the case of rubber-stoppered flasks, a small bore hypodermic needle should be inserted in the serum bottle cap prior to sterilization. A small cotton plug in the exposed end of the needle maintains sterility (FIG. 3). The needle functions as an escape valve to prevent forcing the stopper out of the flask during autoclaving. In the case of cotton-stoppered flasks, the inoculating tube plugged with a serum bottle cap is affixed to the side of the flask (FIG. 2).

The method of harvesting consists of tipping the culture flask so that the beads will fall out of their container, and then shaking the flask vigorously. Sterile water or liquid medium is injected into the flask through the inoculating tube, and the vessel agitated gently. By shaking the flask the released glass beads scrape the colony surface and dislodge the conidia. Aliquots of the resulting conidial suspension are drawn out aseptically with a hypodermic needle inserted into the inoculating tube.

With this technique the scraping device is sterilized in the culture



FIGS. 1–4. Glass bead (pellet) container. 1. Pellet container showing details of construction. 2. Pellet container positioned in a cotton-stoppered Erlenmeyer flask equipped with a side arm inoculating tube. 3. Pellet container positioned in a rubber-stoppered Roux flask. 4. Positioning of container above the medium in a Roux flask.

vessel with the medium, and the flask need not be opened either for inoculation or spore harvest.—T. W. JOHNSON, JR. AND GORDON W. TUCKER, Chemical Corps, United States Army, Washington 25, D. C.

MYCOTYPHA MICROSPORA FROM MARYLAND[1,2]

This interesting and rare phycomycete was found on an uninoculated glucose-peptone agar plate in our laboratory in July, 1953. A check of the literature and correspondence with Professor V. M. Cutter, Jr. and Dr. C. W. Hesseltine indicates that this is the fourth report of *Mycotypha*. Comparison of our isolate with a culture of Martin's isolate (NRRL-*684*), supplied by Dr. Hesseltine, revealed no significant differences. When both were grown on the same plate of glucose-peptone agar at room temperature (about 26° C) there was no indication of zygospore formation.

Fenner (Mycologia **24**: 187–198. 1932) based the genus and species on a plate contaminant found during an attempt to isolate an orange pathogen in Washington, D. C. Cherry (Mycologia **26**: 133–134. 1934) separated it from a mixed culture on potato-dextrose agar during attempts to isolate a pathogen of flowering quince fruits from the vicinity of Lafayette, Indiana. Martin (Iowa Acad. Sci. Proc. **46**: 89. 1939) reported it from a plate culture over which a fructification of *Calocera cornea* on white pine wood had been hung. This had been collected in Pine Hollow, Dubuque County, Iowa, in October, 1938.

It would be interesting to learn the natural habitat of this organism. Perhaps the sensitivity of the fungus to high concentrations of hydrogen ions could be responsible for its seeming rarity, since many routine mycologic media are adjusted to an acid pH. Although our isolate grows best on an organic source of nitrogen, it does not require vitamins in the medium, nor does it need any specific amino acid.

Dr. Hesseltine now has cultures of all of the isolates of *Mycotypha microspora* except Fenner's original one, which he would be pleased to obtain.—JOHN L. WOOD, Fungus Laboratory, The Johns Hopkins Hospital, Baltimore 5, Maryland.

A REVISION OF THE GENUS KEITHIA

Keithia Sacc. is a genus of Discomycetes parasitic in the leaves of conifers. Three species were treated by E. J. Durand (Mycologia **5**: 6–11. 1913) and two additional species have been described, one by

[1] Contribution from the Department of Medicine, School of Medicine and the Department of Microbiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore 5, Maryland.

[2] This investigation was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract Number DA-149-007-MD-153.

J. F. Adams (Torreya **18**: 157–160. 1916), and one by J. K. Miller (Jour. Elisha Mitchell Sci. Soc. **51**: 167–171. 1935). This last, *K. juniperi* Miller, differs primarily from the other four species according to its author in having 8-spored, rather than 2- or 4-spored, asci. He writes (*loc. cit.*, p. 171): "The morphology of the pathogen on cedar is quite like other known species of *Keithia*, except that it has eight spores." Miller also described a two-walled ascus in this species, but apparently little significance was attached to this character.

The presence of two-walled (bitunicate) asci in a Discomycete would be an exception to Luttrell's (Univ. Missouri Studies **24**(3): 1–120. 1951) concept that this character is restricted to the loculate Pyrenomycetes, and offers a serious problem in the taxonomy and phylogeny of Ascomycetes. For this reason, a re-examination of the described species of *Keithia* was undertaken at the suggestion of the junior author. Specimens were examined from the Department of Plant Pathology Herbarium at Cornell University and from the New York Botanical Garden (through the courtesy of Dr. D. P. Rogers).

All the species examined, with the exception of *K. juniperi*, were found to be essentially as described by Durand and Adams. All four species proved to be Discomycetes, with the fleshy apothecium developing beneath the host epidermis and rupturing it at maturity, splitting it lacinately or throwing it back as a scale or flap. The excipulum and hypothecium are poorly developed.

In *K. juniperi*, however, the carbonaceous ascocarp develops above the epidermis and is exposed to the air from the beginning. Hyphae growing between the epidermis and the palisade layer penetrate to the surface and produce the fruitbody upon a narrow base. At first the ascocarp consists of stromatic tissue, soon differentiating into a more or less hyaline interior and a dark-walled outer layer or layers of cells. This outer layer of cells persists to maturity of the fruitbody, and is doubtless what Miller referred to as the "excipulum."

The four typical species of *Keithia* have unitunicate, cylindric to clavate asci lying parallel to one another and forming a palisade layer, and are interspersed with apically free, true paraphyses. (A palisade layer of paraphyses is formed before the asci appear.) In *K. juniperi* the asci are broad-clavate and bitunicate (the outer wall ruptures and the inner extends before liberating the spores). The asci arise from a more or less hemispherical stromatic cushion at the base of the ascocarp and diverge radially outwards. Two types of interthecial threads were observed: narrow, thin-walled, hyaline threads (pseudoparaphyses?) and broader, thick-walled, brown threads (remnants of the locule walls?).

The latter type of thread is illustrated by Miller, who terms them paraphyses. Our sections have shown that the thick-walled threads tend to branch and anastomose, and certainly merge into the darkened outer layer of the stroma. In sectioning, both types of threads were often apparently broken at or near the upper point of attachment, giving the illusion of apically free paraphyses as described by Miller. This artifact doubtless led him to describe the ascocarp as an apothecium.

Concluding that *K. juniperi* is not a Discomycete, it was felt desirable to place it in its proper taxonomic position. A specimen of *Dothidea sphaeroidea* Cooke on *Juniperus virginiana* (Ravenel, Fungi Americani *387*) was found to be essentially identical with the type specimen of *K. juniperi,* and both specimens match the description given by Theissen and Sydow (Ann. Myc. **13**: 149–746. 1915) under the name *Coccodothis sphaeroidea* (Cooke) Theiss. & Syd. It is our belief that *K. juniperi* should be treated as a synonym of *C. sphaeroidea,* which would be placed in the Pseudosphaeriales by modern taxonomists.

The removal of *K. juniperi* from the Discomycetes removes one obstacle to the acceptance of Luttrell's classification. The remaining species of the genus are all closely related, and each appears to be restricted to a single genus of host plants. Two species have 4-spored asci: *K. tetraspora* (Phil. & Keith) Sacc. on *Juniperus* in Europe; *K. tsugae* (Farlow) Farlow *in* Durand on *Tsuga* in North America. Two species have 2-spored asci: *K. thujina* Durand on *Thuja; K. chamaecyparissi* Adams on *Chamaecyparis,* both known only from North America. The first three species were well described by Durand (*op. cit.*) and the last by Adams (*op. cit.*). The major point overlooked by these workers is that the ascospore wall in all four species is characteristically pitted, a condition only noted by them in *K. thujina,* in which the marking is very pronounced.—MARIA E. PANTIDOU AND RICHARD P. KORF, Department of Plant Pathology, Cornell University.

REVIEWS

MANUAL OF THE NORTH AMERICAN SMUT FUNGI, by George William Fischer. 343 pp., 136 figs. The Ronald Press Company, New York, 1953. Price, $8.75.

Since 1859 most taxonomic arrangements have been designed both to reflect phylogenetic speculation and to catalogue, a duality that has, for applied mycologists at least, only too often bedevilled manuals for the identification of fungi. One of the merits of the present work is the uncompromisingly practical approach of the author who has arranged the 276 species of smut fungi recorded for North America in one strictly alphabetical series from *Burillia acori* to *Ustilago williamsii*. This list is integrated by an adaption of Clinton's key to genera (which dispenses with spore germination as a diagnostic character), a specific key for each genus, a generic host index in which the species attacking any genus are, when necessary, again keyed out, and, in conclusion, a general index to all the host and fungus names, including synonyms of the latter.

Each species is treated according to a common plan. After the accepted name and any synonyms, there is a concise description of the smut (firmly based on the examination of specimens), its hosts (in alphabetical order), information on the North American distribution, a photomicrograph of the spores, usually at a magnification of × 900, and often a habit photograph.

As could be expected from a knowledge of Professor Fischer's many papers on the smuts, a broad morphological species concept is adopted but, when convenience is at stake, this approach is never taken to its logical conclusion and as a guiding principle morphologically similar smuts which occur on host plants belonging to different families are distinguished by different binomials.

Given details of a smut fungus recorded for North America, or, even more easily, with less knowledge of the fungus but with the host plant correctly identified, the possessor of this eminently usable volume should find little difficulty in identifying the parasite.

Though primarily intended for use in the United States and Canada, where its worth will be most fully appreciated, there are many mycologists in other parts of the world who will have occasion to consult this Manual, which maintains the high standards of American mycological scholarship.—G. C. AINSWORTH.

THE POLYPORACEAE OF THE UNITED STATES, ALASKA, AND CANADA, by L. O. Overholts. Prepared for publication by Josiah L. Lowe. xiv + 466 pp. Portrait and 132 pls. University of Michigan Press, Ann Arbor, 1953. Price, $7.50.

At the time of his death in 1946, Dr. Overholts had been working for 23 years on this manual, revising it from time to time, and he left it in substantially the form in which it now appears. It thus represents the summation of over half a century of work on this important group of fungi. Dr. Lowe has prepared it for publication, brought the bibliography, the keys and the notes on distribution up to date and added notes on about 30 species and varieties which had been omitted, these last inserted after the systematic treatment of the genera to which they would be assigned in Overholts' classification.

Overholts' extreme conservatism in recognizing genera is well known, and is defended in the introduction, pp. 4–10. He includes all polypores from North America north of Mexico in the ten genera *Cyclomyces, Merulius, Poria, Fomes, Daedalia, Lenzites, Hexagona, Favolus, Trametes* and *Polyporus*. *Merulius* and *Poria* are not treated in the present volume, *Hexagona* includes but a single species, and Gilbertson has recently shown (paper read September, 1952, at the Ithaca meeting of the A.I.B.S. and published in Mycologia **45**: 229–233. 1954) that *Cyclomyces Greenei* is merely a variant of *Polyporus Montagnei*. This leaves six genera to accommodate the 233 additional species recognized as well as those of the 30 added by Lowe which are believed to be well-founded. *Echinodontium* is not mentioned, presumably because its position in the Hydnaceae is regarded as well-established. The book is beautifully illustrated with 675 photographs reproduced on 124 plates and 181 drawings of microscopic features on 8 additional plates. A glossary and index complete the volume. The descriptions are clear and as concise as is consistent with precision and completeness and the keys appear to be workable.

It seems improbable that the extremely broad generic concepts employed will be acceptable to modern mycologists, although they will undoubtedly be welcomed by the numerous practical workers who are interested in these fungi as wood-rotters. It may also be argued that the Polyporaceae, even as here restricted, is an unnatural aggregation of species grouped together on the basis of hymenial configuration, which is itself a character of subordinate significance. Corner, in a recent paper (Phytomorphology **3**: 152. 1953), says: "I believe that the natural classification of the polypores will be the hardest problem in the

systematics of Basidiomycetes." If this is true, and it may well be, perhaps the extremely conservative treatment of Overholts will in the long run be useful to more people than would an attempt, at the present time, to restrict generic concepts, provided, of course, that this treatment is recognized as provisional.

The nomenclature and citations are not always in accordance with the current code. The names selected are all familiar however. The frequent citation of combinations to pre-Friesian authors as "Batsch ex Fries," "Bolt. ex Fries," etc., where the original epithets were published in *Boletus* or *Agaricus* is misleading, but not seriously so.

There is an enormous amount of information in the book, and mycologists everywhere will find it indispensable. They will be grateful to Dr. Lowe for undertaking the task of preparing it for publication and to the publishers for having issued it in such attractive form.—G. W. MARTIN.

A DICTIONARY OF THE FUNGI, by G. E. Ainsworth and G. R. Bisby. Fourth edition. viii + 475 pp., 138 figs. Commonwealth Mycological Institute, Kew, Surrey, England, 1954. Price, 20 shillings (about $3.00).

The earlier editions of this work have established its right to a place on a convenient shelf in every laboratory where fungi are seriously studied. The remaining stock of the third edition, published in 1950, was destroyed by the floods of the winter of 1953 and it was wisely decided not to reprint that edition from standing type, but to bring it up to date. This included the incorporation of 525 new genera, many, as the authors comment, "doubtless unnecessary," but in the literature and therefore subject to consideration. Equally important is the inclusion of changes resulting from the application of the International Code as adopted at Stockholm. The fourth edition contains 475 pages as compared with 447 in the third edition, but the incorporation of new material may be observed on a very large proportion of the pages. There is no other work which can take the place of this book and the authors and the Commonwealth Mycological Institute have rendered a distinct service in making it again available and as nearly contemporary as is possible in an undertaking of this sort.

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